Henry B. Morrison Jr

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Kansas State Agricultural College team, winner of All-Products Sweepstakes, 14th Students' National Contest in Judging Dairy Products, Cleveland, October 21, 1930. Reading left to right: W. W. Babbit, Ralph F. Germann, Professor W. H. Martin, J. L. Wilson, W. J. Braun.

Veni, Vidi, Vici

THE team from Kansas State Agricultural College came, saw and conquered at the 14th Students' National Contest in Judging Dairy Products in Cleveland last year. Will a Kansas team repeat this victory at Atlantic City next month? Or will some other team, **yours** perhaps, have its name broadcast the country over as the winner?

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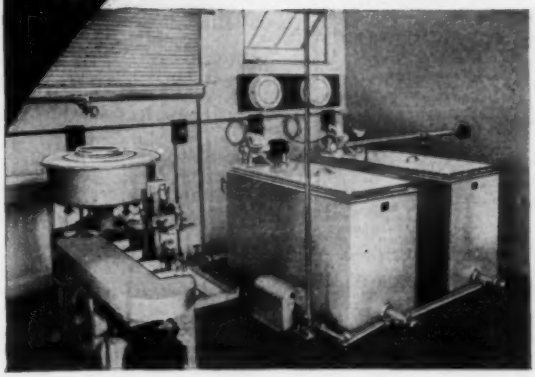

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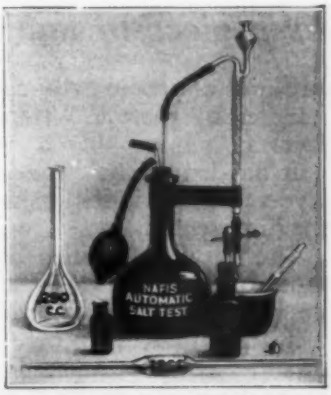

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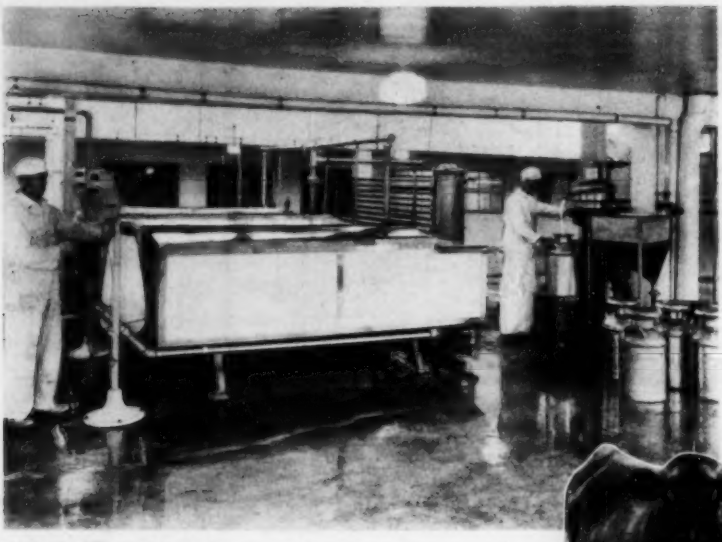

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THE CONTRIBUTION OF THE DAM IN INHERITANCE OF MILK AND BUTTERFAT*

LYNN COPELAND

Register of Merit Department, American Jersey Cattle Club, New York City

Raising the level of the average production of dairy cattle in America has in the past been accomplished primarily through improved feeding methods, better management, and through the elimination of low producing animals. Obviously, the degree to which the average production can be increased by these methods is limited. The results of Register of Merit and Herd Improvement Association testing during the past five years indicate that little further progress is being made by this means. To continue to raise this level of production it seems apparent that it is to inheritance and scientific breeding methods that cattle breeders and dairymen must look.

Modern breeding methods depend primarily on the selection and continued use over a period of time of prepotent herd sires. Genetic research has, during recent years, centered on methods of locating and choosing such sires. The herd sire has been given prominence by investigators since his offspring are far more numerous than those of any one cow, and he contributes one-half of the inheritance given to them. In much of the recent literature on the subject of inheritance of milk and butterfat production, the influence of the dam is almost ignored. Certainly the literature emphasizing the contribution of the dam from a genetic viewpoint is meagre compared to the amount that has been written relative to the sire. This is due not only to the greater number of progeny of the sire, but also because it is more readily possible to determine a bull's transmitting ability by the performance of his daughters.

Yet, judging from progress now being made it will be many years before any appreciable percentage of dairy calves are sired by proved bulls. In any event a great number of unproven young

* Received for publication February 11, 1931.

bulls must always continue to be used. Any proven bull was once an untried youngster and some breeder had to take a chance in proving him. It is a tragedy to the industry that so many of these good young prospective herd improvers are failures when proven. It is a challenge to constructive breeders and students of breeding to uncover additional facts and supply more information to dairymen that will materially assist them in selecting untried bull calves, calves that will in the majority of cases prove out successful.

A study of the influence or contribution of the dam involves a number of questions. The first of these is, just what is the importance of a production record of a cow? Has it any value from a breeding standpoint other than only indicating the transmitting ability of the cow's sire? When selecting an unproven sire, it is still the almost universal practice to give a great deal of consideration to the production record of the dam. Bull calves from dams with records over 1,000 pounds of butterfat are invariably in demand. This is because of the hope that the damwill transmit a measure of her own high producing ability to her son and through him to her granddaughters. Does this theory work out in actual breeding practice or is the percentage of failures so great as to render it of doubtful value? McDowell's and Parker's tabulations (1) show that as the production of the dams advances, the production of the daughters gradually climbs, but more slowly. This would indicate that there is at least a slight degree of relationship between the record of the cow and the records of her progeny. If it is true that the production record of a cow furnishes a fairly reliable measure of her transmitting ability, can it be assumed from this that there is a lack of dominance and that duplicate genes have a cumulative effect in inheritance of milk and butterfat production.

The theory has also been advanced that some of the hereditary factors governing milk and butterfat production are sex-linked and that possibly the dam should be given more consideration in the selection of a future herd sire. These and a host of other questions are involved in attempting to formulate any opinions on the ultimate value of a cow's record in the serious business of con-

structive cattle breeding. In view of the vital importance of the subject of increasing production it is the object of this study to present further data on the contribution of the dam and to determine if possible what conclusions might be drawn as to the relationship between her own producing ability and the producing ability of her daughters and granddaughters.

METHODS AND DATA

The materials used in this investigation were the records in the Register of Merit Department of the American Jersey Cattle Club. Since the establishment of test work nearly thirty years ago, over forty thousand long-time production records have been completed by Jersey Cows. The data on each of these records are still available in the files of the Register of Merit Department. In checking through these files it was found that to June 1, 1930, there were 694 cows that had production records themselves and also had at least one tested daughter and one Register of Merit son. (A son to be in the Register of Merit must have at least three tested daughters from different dams.) The highest records of each of these cows were converted to a mature 365 day basis. In doing this the records were first computed to a 365 day basis using the factor of 1.15 previously worked out in the Register of Merit Department (2). The records were then figured to a mature basis through the use of the age factors published by Fohrman (3). Likewise, the records of the daughters were converted to a uniform age basis. When the cow had two or more tested daughters, the records of the daughters were of course averaged. Similarly, the highest records of the sons' daughters were computed to a mature 365 day basis and then averaged.

When this preliminary work was completed, the records of the dams were compared with those of their daughters and with the records of their sons' daughters. The relationship or correlation between the dams' records and the sons' daughters' production will give an indication of the value of a production record in indicating or measuring the transmitting ability of the dam. The results of these comparisons are shown in tables 1 and 2 and

TABLE 1

Frequency table comparing records of dams and daughters

FAT PRODUCTION DIVISIONS OF DAMS	NUMBER OF COMPARISONS	AVERAGE YIELD OF DAMS	AVERAGE YIELD OF DAUGHTERS	DISTRIBUTION OF DAUGHTERS (POUNDS OF FAT)										
				Over 900	800–900	750–800	700–750	650–700	600–650	550–600	500–550	450–500	400–450	Below 400
pounds		*pounds*	*pounds*											
Over 900	26	959.86	678.00	1	4	1	3	6	4	2	4	1	0	0
800–900	54	844.63	683.15	2	6	6	9	11	6	8	4	2	0	0
750–800	39	772.81	600.02	0	1	1	5	5	7	6	7	6	1	0
700–750	55	721.85	616.24	0	3	1	2	13	15	10	4	4	2	1
650–700	77	671.63	596.23	1	7	3	4	10	8	7	19	12	5	1
600–650	77	625.44	583.35	1	1	5	4	15	6	14	9	10	8	4
550–600	69	578.66	558.34	1	0	6	3	3	7	11	14	12	9	3
500–550	93	525.46	544.25	0	1	1	3	11	10	15	20	15	11	6
450–500	84	479.10	516.14	0	0	1	2	5	6	10	21	19	13	7
400–450	73	431.86	532.98	0	1	1	3	6	7	7	18	14	12	4
Below 400	47	379.63	502.00	0	0	1	1	3	3	4	7	13	9	6
Totals...	694	603.30	572.97	6	24	27	39	88	79	94	127	108	70	32
Percentage distribution...				0.9	3.5	3.9	5.6	12.7	11.4	13.5	18.3	15.6	10.1	4.6

TABLE 2

Frequency table comparing records of dams and records of sons' daughters

FAT PRODUCTION DIVISIONS OF DAMS	NUMBER OF COMPARISONS	AVERAGE YIELD OF DAMS	AVERAGE YIELD OF SONS' DAUGHTERS	DISTRIBUTION OF SONS' DAUGHTERS (POUNDS OF FAT)										
				Over 900	800–900	750–800	700–750	650–700	600–650	550–600	500–550	450–500	400–450	Below 400
pounds		*pounds*	*pounds*											
Over 900	26	959.86	615.56	0	0	1	1	5	11	5	2	1	0	0
800–900	54	844.63	588.18	0	2	2	4	5	10	13	12	6	0	0
750–800	39	772.81	578.95	0	0	1	2	3	6	16	6	5	0	0
700–750	55	721.85	573.91	0	0	2	3	7	3	17	13	8	2	0
650–700	77	671.63	549.46	0	1	0	0	4	14	15	23	16	4	0
600–650	77	625.44	535.98	0	0	0	0	4	11	19	19	16	8	0
550–600	69	578.66	577.58	0	0	3	5	3	11	18	16	11	2	0
500–550	93	525.46	548.72	0	0	1	3	3	15	19	28	14	8	2
450–500	84	479.10	524.45	0	0	0	3	1	9	12	24	27	5	3
400–450	73	431.86	514.85	0	0	0	0	0	4	14	23	25	5	2
Below 400	47	379.63	488.62	0	0	0	0	0	4	2	9	20	8	4
Totals..	694	603.30	547.94	0	3	10	21	35	98	150	175	149	42	11
Percentage distribution...					0.4	1.4	3.0	5.0	14.1	21.6	25.2	21.5	6.1	1.6

figure 1. These tables also show the distribution of the records of the daughters and the records of the sons' daughters.

Examination of these tables and the accompanying figure shows that as the dams' records increase the production records of the daughters also increase and likewise the yield of the sons' daughters show some increase. However, the increase is much less relatively than that of the dams. The average production of all the mature (6 to 10 years) 365 day Register of Merit records that

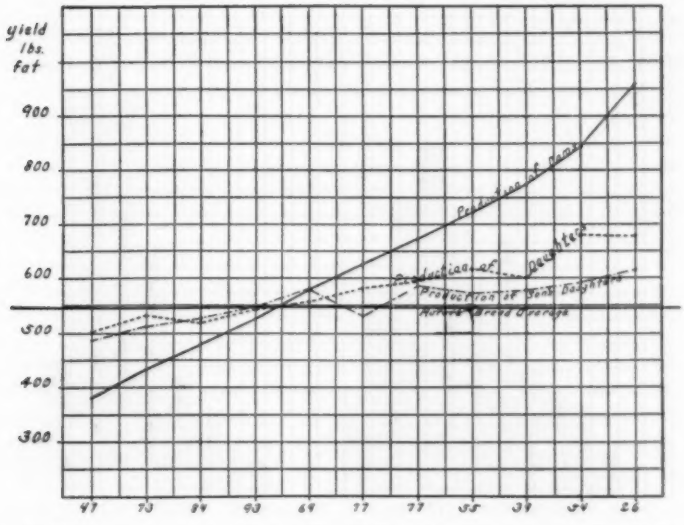

FIG. 1. COMPARISON OF RECORDS OF DAMS, DAMS' DAUGHTERS AND DAMS' SONS' DAUGHTERS

have ever been completed is 547.52 pounds of fat. This is shown by the horizontal line in figure 1. It should be noted that the average production of all of the sons' daughters is in almost exact agreement with the breed average. Comparing the records of the dams and daughters with the breed average it was found that for every pound the dams varied from the breed average the daughters varied about 0.32 of a pound, that is, when the dams were 100 pounds above the breed average, the daughters were approxi-

mately 32 pounds above the average. On a percentage basis the daughters showed 32 per cent as much variation from the breed average as did their dams. The frequency distribution of the records of the daughters and of the sons' daughters is shown much more clearly in figure 2.

It will be noted from the shape of the curve in the frequency polygon that the minimum entrance requirements for the Register of Merit exclude a significant number of the daughters and sons'

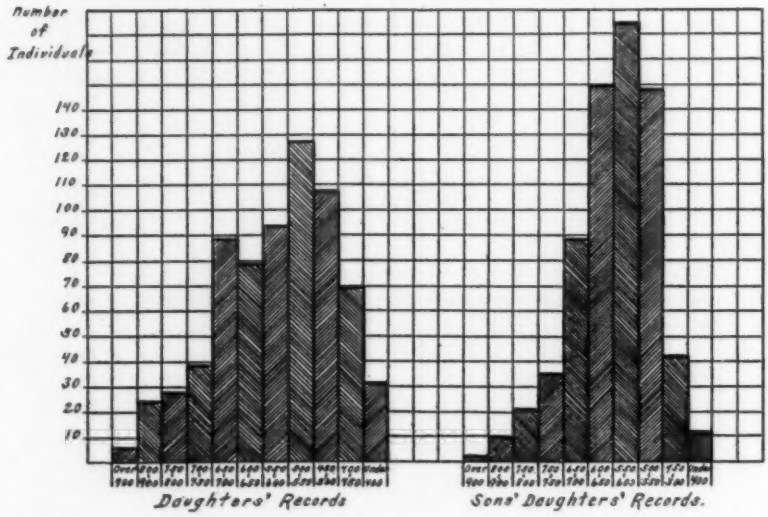

FIG. 2. FREQUENCY POLYGON SHOWING PRODUCTION DIVISIONS OF DAMS' DAUGHTERS AND DAMS' SONS' DAUGHTERS' RECORDS

daughters. This undoubtedly effects the direction of the daughters' curve and the sons' daughters' curve in the graph where the dams are below 550 pounds of fat and of course effects the coefficients of correlation between the production of the dams and the yield of their daughters and sons' daughters. If all the daughters and sons' daughters that failed to qualify were shown, the degree of correlation would be higher. Table 3 shows these constants for dams and daughters and dams and sons' daughters.

From table 3 it will be seen that the coefficients of correlation between the dams and daughters and dams and sons' daughters while not extremely high are nevertheless very significant. Gowen (4) working with Holsteins reported a slightly higher correlation, (0.497 ±0.021) between dams and daughters and less correlation, (0.307 ±0.047) between maternal grandams and granddaughters. There are several reasons why the correlation between the dams and daughters should vary from that of the dams and sons' daughters. In fact, it is surprising that the variation is not greater than it is. One important reason is that the daughters of these cows were in the majority of cases tested in the same herds in which their dams were tested, while the daughters of the sons are more likely to be tested in different herds and

TABLE 3

Physical constants for dams and daughters and dams and sons' daughters

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	CORRELATION BETWEEN DAMS AND DAUGHTERS	CORRELATION BETWEEN DAMS AND SONS' DAUGHTERS
Dams	603.30±3.89	151.88±2.75	2.52±0.046		
Daughters	572.97±3.11	121.29±2.19	2.12±0.038	0.4044±0.0278	
Sons' daughters	547.94±2.21	75.50±1.56	1.38±0.029		0.3415±0.026

under different conditions. The daughters of the sons have only about 25 per cent of their inheritance that would come through the dams while 50 per cent of the daughters' inheritance would come through the dams. Then too, these dams were probably rarely mated with sires possessing the same level of inheritance as they themselves had, consequently, even though the dams were homozygous, for their level of production the offspring are not likely to secure from their sires an inheritance for the same level of production as they do from their dams. In the case of the granddaughters this chance for an inheritance for a different level of production is introduced twice, not considering the possibility of the sons' dams being heterozygous, but considering only the chance of inheritance of different levels of production being introduced through the sons' sire and the sons' daughters' dams.

There is also a tendency for the better dams to be mated with better sires and the poorer dams with poorer sires.

While the correlation between the dams' records and the records of the daughters is undoubtedly somewhat higher than would be obtained were it possible to hold the influence of the sires fairly constant, on the other hand the elimination of low producing daughters through failure to meet the minimum entrance requirements tends to lower the degree of correlation that was found.

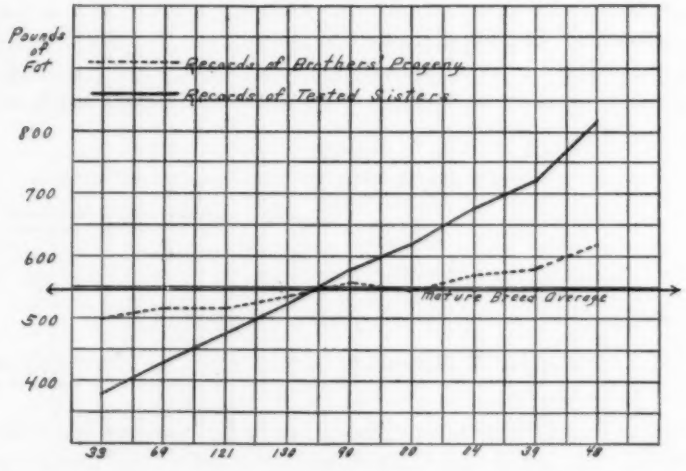

FIG. 3. RECORDS OF SISTERS COMPARED WITH RECORDS OF THEIR BROTHERS' DAUGHTERS

The data do indicate that the dams had to rank close to 600 pounds of butterfat in order to raise the production of their daughters above the mature breed average of 547 pounds while they had to average better than 700 pounds of butterfat in order for their sons' daughters to exceed the average mature production of the breed. It is evident that a cow's record is nearly twice as reliable a measure of the production of her own daughters as it is of her sons' daughters.

It was thought possible that a better index of the dam's ability

to transmit production to her granddaughters through her sons would be the average production of all of her tested daughters. In other words, is it worth-while in selecting an unproven sire to give consideration to the records of his maternal sisters? In view of this a comparison was made between the records of the dams' daughters and the dams' sons' daughters. While it was not determined for the entire number, cursory examination revealed that in approximately 50 per cent of the cases the tested sisters were by the same sire as the proven son, and in the other 50 per cent the sisters were by different sires. No attempt was made to segregate the half-sisters from the full-sisters. Figure 3 shows the results of this comparison between the records of the tested sisters and the records of their brothers' progeny. The coefficient of correlation is shown in table 4.

TABLE 4

Physical constants for sisters' records and records of brothers' progeny

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	CORRELATION BETWEEN SISTERS AND BROTHERS' PROGENY
Sisters' records	572.97±3.11	121.29±2.19	2.12±0.038	0.3679±0.025
Records of brothers' progeny	548.03±2.22	75.77±1.57	1.38±0.029	

It is surprising that the correlation between the records of the dams' daughters and the records of their sons' daughters is but little higher than that between the dams' own records and the records of their sons' daughters. For explanation, however, it may be added that in 131 instances the son had three or more tested sisters, in 196 cases there were two tested sisters, and in the remaining 367 instances there was only one tested sister. Davidson (5) concludes that it is necessary for a bull to have at least six unselected tested daughters in order to approximate his transmitting ability, more is very desirable. In view of this, it was not expected that the amount of correlation shown in table 4 would be very high. To date less than 300 Jersey cows are dams of four or more tested daughters and only 95 have five or more tested daughters. Nearly all of these dams are dead and hence an

attempt to measure the inheritance or transmitting ability of a cow through a progeny performance test holds little possibilities.

Yet another indication of a cow's transmitting ability may lie in the production of her own tested sisters. To study the reliability of this index the records of the dams' paternal sisters were all converted to a mature 365 day basis. These records were then added to the dam's own record and the average determined. A better explanation might be that the average production of the dams' sires' daughters was computed. In 152 instances the dam either had no tested sisters or only one tested sister. Only the remaining 542 cases where the dam had at least two tested sisters were used in this analysis.

TABLE 5

Physical constants for dams' sisters, daughters and sons' daughters

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	CORRELATION BETWEEN DAMS' SISTERS AND DAMS' SONS' DAUGHTERS	CORRELATION BETWEEN DAMS' SISTERS AND DAMS' DAUGHTERS
Dams' sisters..	531.34±2.02	60.89±1.43	1.15±0.027		
Dams' daughters.....	576.86±3.92	118.39±2.77	2.05±0.048		0.4046±0.028
Dams' sons' daughters...	549.30±2.66	80.36±1.88	1.46±0.034	0.3440±0.029	

At first the individual dam's record was compared with the average record of all of her sire's daughters, (her own record included). The correlation coefficient was found to be 0.561 ± 0.022. The mean fat production of the dams was 603.30 ±3.89 while that of her sire's daughters was 531.34 ±2.02 showing a difference of 71.96.

The average production records of the dams' sires' daughters were then compared both with the dams' daughters and also with the dams' sons' daughters. The results of this comparison are given in figure 4 and table 5.

In examining table 5, it is worth-while and significant to note how closely the coefficient of correlation between the dams' sisters (their own records included) and the dams' daughters compares with the correlation between the dams' own record alone and the

records of her daughters. The same close degree of correlation also exists between the records of the dams' sisters (their own records included) and the dams' sons' daughters and the correlation between the dams' records alone and the records of the sons' daughters. (Refer to tables 3 and 5.) This is not in exact agreement with Turner's findings (6) in which he reports that the relationship between the dam's sire's daughters and the dam's

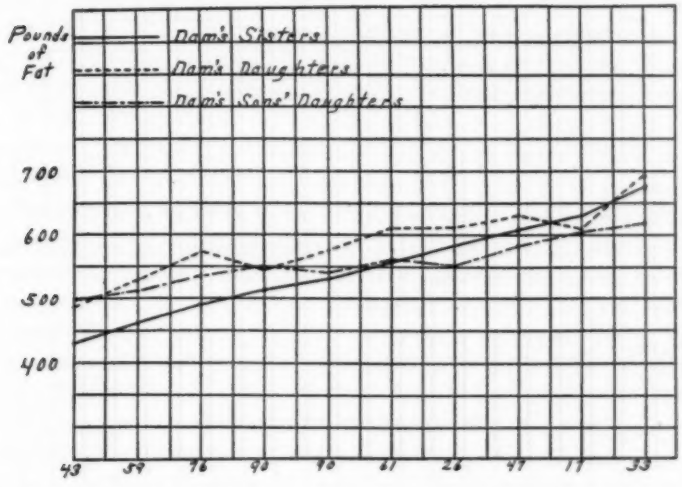

FIG. 4. COMPARISON OF RECORDS OF DAMS' SISTERS WITH DAMS' DAUGHTERS AND DAMS' SONS' DAUGHTERS

son's daughters is much better than the relationship between the dam's own record and the production of her son's daughters.

In reviewing the foregoing tabulations it will be noted that in predicting the producing ability of a bull's daughters the bull's dam's record, the records of a few maternal sisters, and the records of his dam's sisters all rank about equal. This led to the thought that possibly by considering all three of these factors a more reliable indication of a bull's probable transmitting ability might be obtained. To develop this possibility the average production of the dam's daughters and the average yield of the dam's

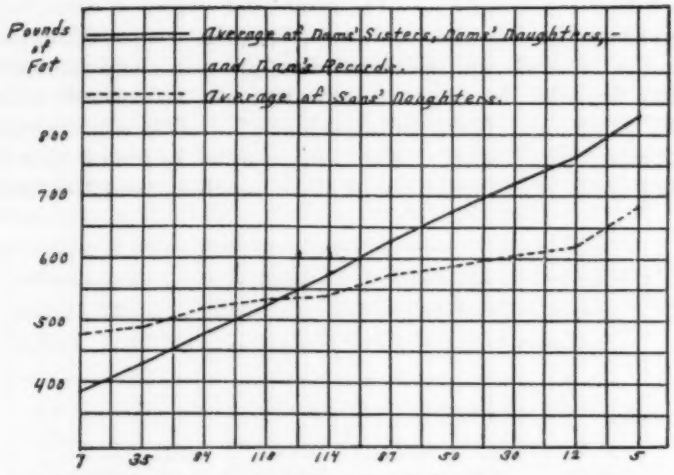

FIG. 5. COMPARISON OF AN AVERAGE OF DAMS' RECORDS, DAMS' SISTERS AND DAMS' DAUGHTERS' RECORDS WITH RECORDS OF DAMS' SONS' DAUGHTERS

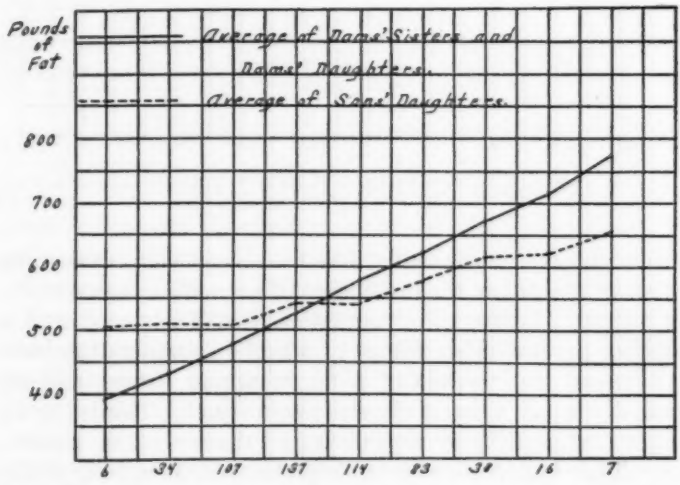

FIG. 6. COMPARISON OF AN AVERAGE OF DAMS' SISTERS' AND DAMS' DAUGHTERS' RECORDS WITH RECORDS OF DAMS' SONS' DAUGHTERS

sire's daughters were averaged with the dam's own record. The average figure thus obtained was compared with the average records of the sons' daughters. A total of 542 comparisons were made, the cases in which the dams had no tested sisters being excluded. The coefficient of correlation between the average figures obtained by the foregoing method and the sons' daughters' records was found to be 0.412 ± 0.027. This degree of correlation is considerably higher than any of the previous comparisons made with the bulls tested progeny. Figure 5, also shows this graphically. This correlation emphasizes that consideration of not only the dam's record but also the average yield of her sisters and the production of her daughters is very advisable in selecting an untried young bull. Of course, it has been shown (7) that equal or more attention should also be given to the sire but a discussion of the sire's contribution was not a part of this study. The results of this comparison suggested and prompted a similar comparison but omitting the dam's own record. That is, the average production of the dam's sire's daughters was added to the average production of the dam's daughters and the result divided by two. The average figure obtained was then compared with the sons' daughters' records, 542 comparisons being made. In this case the coefficient of correlation was 0.409 ± 0.028. This is nearly as good correlation as was secured when the dam's record was included. Figure 6 indicates the relationship.

SUMMARY

The significance of these results to practical breeding may be summarized as follows:

1. It is evident that there is some correlation between a cow's producing ability and the producing ability of her daughters. Selection of females from high record dams should result in a herd that will produce in excess of the breed average. In general the daughters show about 32 per cent as much variation from the breed average as do the dams.

2. There is a slight correlation between the dam's record and the records of her sons' daughters. However, it appears that very little progress can be hoped for if sires are selected solely

on the basis of their dam's records. It is obvious that a production record considered by itself is a poor index for the germinal composition of the dam for the characters for production. The record of the dam is nearly twice as reliable a measure of the production of her own daughters as it is of her sons' daughters.

3. The records of only a very few tested maternal sisters does not alone furnish a reliable guide for predicting the transmitting ability of an untried bull.

4. There is some correlation between the average production of the dam's half-sisters by the same sire and the average production of the dam's sons' daughters. However, selecting a bull with but this in mind is not recommended from the results of this investigation.

5. In choosing any untried young bull consideration should not be limited to the sire alone but the maternal ancestry is worthy of careful study. *Too much information cannot possibly be obtained.* The results of the last phase of this investigation indicate that when an average is made of the dam's record, the dam's sisters' records and the dam's daughters' records the average figure obtained in this manner provides a more accurate and reliable index as to a bull's probable transmitting ability than does any one of these factors considered by itself. If the dam has no record the average of her maternal sisters' records and of her daughters' records gives just about as dependable results.

6. In purchasing an untried young bull it is now generally recommended, in the light of present knowledge, that first of all he be sired by a proven bull, a bull whose daughters are uniformly high producers and superior to their dams. After this fact has been verified attention should then be given not only to the dam's record but also to the records of her sisters and the records of her daughters. Selection of a bull by this method seems to provide the most possibilities for success.

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UNIFORM RULES FOR THE HERD TEST*

The American Dairy Science Association at its summer meeting ast June at Ames, Iowa, considered and adopted the recommendations of its Breeds Relation Committee regarding uniform herd test rules in connection with the new Herd Improvement Tests. The Uniform Herd Test Rules were developed by the special committee of the Official Testing Section after several special meetings had been held with representatives of the different breed associations. The purpose of the Uniform Herd Test Rules is stated as follows:

1. To standardize and simplify the methods of obtaining and supervising herd tests for economical production of dairy cows of all breeds.
2. To encourage mass testing of dairy herds under herd conditions.
3. To make the records more reliable and more easily comprehended and interpreted.
4. To make possible recognition and greater use of worthy dairy herd sires through production records of all daughters.

The following are the suggested rules:

Entering herds on test. Any owner of registered cattle of a dairy breed may make application at any time to the National Breed Association concerned to enter his herd on test, using forms furnished by the breed association. All cows of milking age, both registered and grade, must be included in the herd test.

Identification of cows on test. All cows on test must be identified by tattoo number or by color sketches on broken-colored animals. The day, month, and year of birth must be reported on all registered animals, and on grades if possible; if not, approximate dates for grades will be accepted.

Conduct of test. The herd test may be started at the first of any calendar month. Twelve consecutive periods of twenty-four hours each, approximately one month apart, without preliminary

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milking, or six consecutive periods of twenty-four hours each, approximately two months apart with preliminary milking, shall be required during the testing year, retests and surprise tests not included. The tester shall indicate on his report which system of testing was used. Parts of two consecutive lactation periods of any cow made within the testing year may be included in the yearly average. Credits for milk may begin with the fourth day after calving, but no butterfat test shall be made before the seventh day. The day on which the calf is dropped is counted as the first day. Single samples are to be taken, each sample to represent each milking of each cow during the twenty-four hours of the test period and each tested for butterfat content.

Number of milkings. Cows may be milked not more than three times daily. Not more than two cows shall be milked at one time and the cows must be so placed that the supervisor can observe the milking of the cows continuously. In no case shall more than 48 milkings be supervised in one day—24 cows if milked twice daily, or 16 cows if milked three times daily. The tester's report must show the number of times each cow is milked during the testing period.

Retests. Retests may be required when any herd averages 40 pounds of butterfat per cow in thirty days, or when after the second month after calving any cow produces in excess of the following:

	Lbs. fat in one day
Junior 2-year old	1.7
Senior 2-year old	1.9
Junior 3-year old	2.0
Senior 3-year old	2.1
Junior 4-year old	2.2
Senior 4-year old	2.3
Mature cows	2.5

Whenever a herd, or individual cow, produces more than these minimum requirements, the tester must notify the state superintendent of testing immediately after the completion of the test. If one or more cows in the herd require a retest the state superintendent in charge may require that the entire herd or any portion thereof be retested.

Surprise tests. One or more surprise tests with preliminary dry milking may be made during the year. Surprise tests shall be made by a different supervisor than the one conducting the previous month's regular test on the herd. The expense of one surprise test, if made, and all retests shall be borne by the owner. Any additional surprise tests will be at the expense of the breed association.

Feed cost and value of product. The owner shall coöperate with the tester by furnishing information regarding value of product, rations used, and local values or prices paid for feed. The tester shall ascertain the weight and record the amounts of all feed consumed by the individual cows, as well as the number of days on pasture. Monthly computations of feed, costs, and income shall be made by the tester and copied by him into a herd record book which shall remain in the possession of the farmer. A duplicate copy of this monthly record shall be furnished on demand to the breed association concerned.

Computing yearly and monthly herd averages. In calculating herd averages the production of all registered first calf heifers that have been in milk ten or more months of the herd test year, and all registered cows that have ever freshened and that have been owned in the herd ten months or more of the herd test year, shall be included in the herd average.

In reporting herd averages, a supplementary list shall be furnished showing all heifers and new cows in the herd less than ten months, together with their production.

Dishonest or fraudulent practice. If the state superintendent of testing is satisfied that fraudulent or dishonest practices have been used in the making of herd test records of registered cows, he shall report same to the breed association which may reject or cancel such records.

Matters not covered by rules. Details of supervision which these rules do not specifically cover shall be administered by the state superintendent of official testing.

Revision of rules. Any revision of rules shall be made by a joint committee of the breed associations and American Dairy Science Association. The chairman of the Breeds Relation Com-

mittee of the Official Testing Section of the American Dairy Science Association shall be ex-officio chairman.

E. L. ANTHONY,
Chairman, Official Testing Section,
J. B. FITCH,
Chairman, Breed's Relation Committee.

THE CHURN AS A SOURCE OF MOLDS IN BUTTER*

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Unquestionably, the typical wooden churn found in our creameries today is the most highly contaminated and the most difficult to sterilize of any dairy equipment. Little has been done to determine the actual extent of infection, particularly by making studies of a dissected or dismantled churn to locate the outstanding areas of contamination or foci of infection, especially concerning molds.

Gregory (1), Haglund and his co-workers (2), Macy and Combs (6), James (4), Paraschtschuk (7), Hood and White (3), Lund (5), and Stiritz (8), have indicted the churn as a prolific source of infection of butter.

It was quite evident from a study of the literature that there was an opportunity to make a more intimate study of typical churns, in order to obtain a better idea as to the presence or absence of molds in various places in the churn itself. The following studies were undertaken with this objective in mind.

Two churns were available for study. Both of them had been in regular use in commercial creameries for a period of years and were obtained at the time they were discarded. Churn A was a Giant Disbrow that had been used for about ten years in a creamery where, previous to its removal, there had been experienced considerable difficulty with moldy butter and excessively high mold and yeast counts. Churn B was a Master Dual that had been in daily use at the Minnesota State Experimental Creamery for a period of seven years, during which no particular difficulty with molds or yeasts had been encountered. Churn A was obtained for study in July, 1928, and Churn B in July, 1930.

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METHODS

Churn A. After its arrival at University Farm, it was found that the inner surface showed numerous areas of visible mold of many types. In order to remove as much as possible, the inside of the churn was washed thoroughly and treated with boiling water and steam for several hours. The mechanical parts were then removed and the churn again washed inside and out with boiling water. Following this, the barrel was cross sectioned at each end by means of an ordinary hand saw. This made it possible to gain access to various parts of the churn with little difficulty. The various parts of the churn were then examined, dissected in a variety of ways, and samples taken from every conceivable place. Scrapings were made from all metal parts or exposed places such as bolt heads, sight glasses, ends of rollers or shafts, stuffing boxes, surface bruises, crevices behind stationary parts, gates, etc. The staves were then carefully removed serially, at both ends and from the middle section. Similarly the parts of each drum were separated. By means of a chisel, which was sterilized in an open flame before each sample was taken, blocks of wood were removed from various places in solid wood areas and adjacent stave surfaces. In the latter case, samples of wood were removed to include each quarter inch from the inner churn surface toward the joint of the staves, which was approximately one inch from the surface. Altogether, 230 representative samples were taken. The samples were placed in sterile 4-ounce, aluminum-capped glass jars.

Churn B. Immediately after the last churning was made, this churn was washed and scalded by the routine method employed by the creamery. Samples from various accessible places were taken immediately. The churn was then removed and the various mechanical parts dismantled as far as possible. The following morning samples were taken from various parts of the churn including scrapings from all surfaces, metal parts, roller ends, etc. The rollers and shelves were taken out promptly and appropriate samples taken. Portions of wood from shelves, drum, staves, etc., were then withdrawn with the greatest possible care by means of a flamed chisel. Especial effort was

made to obtain sections of wood at the surfaces of adjacent, representative staves completely around the barrel. Altogether, 73 samples were taken. These were placed in sterile test tubes and stored in ice until ready for examination.

In both studies, a sufficient quantity of sterile water was added to each jar or tube so that the sample was completely immersed. They were allowed to stand in this way overnight. Platings of 1 cc. of the fluid were made and poured with acidulated whey agar in the usual manner. Incubation was for five days at room temperature. No attempt was made to get quantitative results. The mere presence of mold was to be the criterion.

PRESENTATION OF DATA

Churn A. Of the total of two hundred thirty samples taken, one hundred ninety-two carried molds. The wood at the joints between staves at various depths, up to 1 inch from the inner surface of the churn; wood from cracks in barrel, doors, workers, and shelves, whether at the surface or ½ inch below the surface; wood from the sides or ends of shelves; wooden shelf braces; wooden rolls or workers; scrapings from bolts; metal brackets, inner sides of castings over the ends of workers and in the surrounding or underlying wood, etc., were found to carry molds of one sort or another. The joints in the drums were not as heavily contaminated, this probably being due to the fact that the individual pieces are held more firmly together. The fact that the mycelium of the mold apparently penetrates as far as an inch into the joints between staves is important since those areas present a vast total of possible infected foci.

A representative collection of cultures was obtained from the plates prepared from the samples taken from Churn A. These cultures were identified generically as far as possible and were found to represent eighteen different genera, many of which have been reported as having been isolated from butter. The following genera were found: Aspergillus (9 cultures); Alternaria, Monilia, and Penicillium (7 cultures each); Mucor and Sporotrichum (6 each); Hormodendrum (5); Acremoniella, Botrytis, Phoma, and Spicaria (3 each); Glioclardium, Oospora, and

Spondylocladium (2 each); and one each of Cephalosporium, Chaetomium, Sordaria, and Tilachlidium. Fourteen other cultures produced no fruiting bodies and were not identified.

Churn B. Altogether, 73 samples were taken. Only 22 specimens showed any signs of being contaminated with molds and only 5 of these produced more than two mold colonies on plating. These latter included scrapings from débris behind one of the doors, wood from right upper cradle arm, scrapings from the outer side of the left sight glass, bolt in metal band of right drum, and housing of left end of upper roll shaft. The types of molds present were Mucor, Penicillium, Aspergillus, Alternaria, and Hromodendrum. Although one might expect to find considerable numbers of *Oospora lactis*, it was strikingly scarce in Churn A and absent in samples from Churn B.

While this report does not concern itself with yeasts or bacteria in the churn, it might be mentioned that the former were quite consistently present in the great majority of cases and the latter always, sometimes in large numbers.

DISCUSSION

The results presented here demonstrate that a churn that is in bad condition may harbor considerable numbers and varieties of molds that may prove detrimental to the market quality of butter made in it, if such molds are able to develop. On the contrary, if the churn is kept in good condition by daily use and thorough treatment, it need not constitute a very serious menace. Nevertheless, new churns are not always free from mold, as shown by an examination of a churn recently purchased by the University. The types of molds found in the churns were those which often are responsible for the typical moldy butter where discolored areas mar the appearance and market value of the product. The comparative absence of *Oospora lactis*, which is the typical form encountered on plates prepared for routine mold counts leads one to believe that this mold is indicative of the presence of remnants of cream, butter, or buttermilk in the churn, which in turn means that the churn has not been thoroughly cleaned or has not been in daily use.

The presence of mold in joints between staves at depths of ¾ to 1 inch from the inner surface of the churn is quite significant since it demonstrates that the mycelium must continue its foothold in such remote places and when conditions are favorable, extend hyphae toward the surface for fructification to take place. Consequently, when a churn is allowed to remain idle and especially to dry out so that the staves shrink and the creavices open, the opportunity is presented for this situation to arise. Under ordinary conditions, the bands placed upon the barrel of the churn hold the staves tightly together, but long-continued use of the churn may lead to considerable loosening and subsequent establishment of infected foci.

The crevices between the boards in the end of the churn do not appear to be as highly contaminated as other parts of the churn. This may be due to the fact that the boards are held more tightly in place so that less space is left for the introduction of infection.

The excessive strain which is placed upon the churn during the butter working process undoubtedly results in the dislodgment of contaminated material from various areas involved with the moving parts, such as rollers, and particularly about the packing and bolts. It has been common experience to find that the contamination of butter is greater during the working period than it had been while the rollers were idle.

The reason that species of molds other than *Oospora lactis* seldom appear in considerable numbers on plates made for mold and yeast counts is undoubtedly due to the high dilution on the cream and its fractions, butter-buttermilk. The very nature of the scattered discolored areas indicative of moldiness of butter is another demonstration of this fact.

The evidence presented in this paper makes it clear that a churn may be an important source of mold in butter and should receive careful attention by the buttermaker in his methods of sanitation to reduce the contamination from this particular piece of equipment.

SUMMARY

1. Two churns from commercial creameries were studied to determine the presence of mold.

2. Molds of several types were found in various places in both churns, especially in Churn A, which had been known to be giving trouble.

3. The genera of molds present in the churns are listed.

4. It is shown that the churn may be an important source of mold in butter.

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PRELIMINARY STUDIES OF CHURN SANITATION*

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It has been demonstrated clearly that the churn is an important source of contamination of butter. Once the various parts of the churn become infected, it is an extremely difficult task to eliminate the contamination or even to control the situation satisfactorily. The problem of churn sanitation has been before the industry for many years, and no universally acceptable method for controlling this difficulty has been suggested. The studies here reported were undertaken to serve as a basis for further investigations with the objective of finding the most desirable and most satisfactory method of treating churns to remove or control the infected areas.

Previous studies of churn sanitation have shown that no particular method has proven to be most satisfactory. Gregory (2), Lund (6) (7), Shutt (8), Hood and White (4), Haglund, Barthel, and Waller (3), James (5) and workers at the Indiana Station (1), have studied the problem in various ways and have demonstrated that the sanitation of the churn is exceedingly important and likewise difficult to accomplish.

METHODS

In the experiments here reported, preliminary studies were made of various methods of treatment. A No. 2, Dairy Disbrow churn, with a capacity of 90 gallons of cream, which had been idle for some time, was selected for use. There was visible mold growth at various places on the inner surfaces. In addition, broth cultures of *Alternaria humicola*, *Aspergillus niger*, *Aspergillus*

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flavus, Hormodendrum clasdosporioides, Oospora lactis, Penicillium biforme, Penicillium expansum, Mucor sylvaticus, and *Rhizopus nigricans* were introduced into the churn and allowed to grow several days before treatments began. It was desired to contaminate the churn excessively and to permit the molds to spread as extensively as possible so that extreme conditions would prevail. Thereafter, treatments with hot water, steam, hypochlorites, and chloramines were carried out. These methods will be discussed more in detail under separate headings later. In general, before each treatment, the churn was rinsed with three gallons of tap water. Following this rinse, the hot water or chemical solution was placed in the churn. When steam was used, the hose was placed in the churn, the door laid across the hose, and high pressure steam allowed to pass directly into the churn. Following the various treatments, a gallon of autoclaved skimmilk was used to rinse the churn. This material provided a good medium for the growth of any surviving organisms and in the instances where chemicals were used, it provided for the absorption of excess chlorine, thus eliminating any residual effect of the chlorine on the plates prepared from the samples.

Representative samples for plating were taken of the rinse water from the churn before the treatment and of the skimmilk rinse after the churn had been treated. Check samples were also taken regularly of the water, chemical solutions, and autoclaved skimmilk. In a few cases the water and chemical solutions were not absolutely sterile, but not sufficiently contaminated to vitiate results.

The samples were plated directly using whey agar as the culture medium. The medium for the mold plates was acidulated in the usual manner with tartaric acid. The plates were incubated for five days at room temperature.

The results were not recorded quantitatively, but qualitatively, to give a relative idea of the amount of infection. The symbols used in recording the results were as follows: (−) no growth; (+) a few colonies; (++) many colonies; and (+++) very many colonies.

PRESENTATION OF DATA—PRELIMINARY HEAT PENETRATION STUDIES

Inasmuch as hot water has been commonly used for churn treatment, it was considered desirable to make a preliminary investigation of the penetration of heat into wood from a churn.

In the first study, adjacent holes were bored in a representative stave (2 inches thick) of a standard churn, one clear through the stave so that the thermometer bulb was immersed in the water inside the churn, a second to within ½ inch from the surface in

TABLE 1

Rate of heat penetration of churn wood

TIME OF EXPOSURE	TEMPERATURE OF WATER	TEMPERATURE OF WOOD AT VARIOUS DEPTHS		
		½ inch	¾ inch	1¼ inch
minutes	*°C.*	*°C.*	*°C.*	*°C.*
Start	71.2	25.9	25.9	25.6
10	96.8	47.4	30.8	26.4
20	96.8	62.2	41.7	31.3
30	97.6	69.8	50.6	37.9
40	97.2	74.8	57.6	44.7
50	97.5	77.4	62.9	50.3
60	97.5	80.1	66.8	54.5
70	97.3	81.6	69.8	57.8
80	97.1	82.6	71.9	60.3
90	97.4	83.6	73.7	62.3
100	97.3	84.3	75.0	63.8
110	97.5	84.4	75.8	64.8
120	97.7	85.2	76.4	65.7

contact with the water, and a third to within 1 inch from that surface. The churn was filled with water at 80°C. and kept at that temperature for two hours. At the end of this period, the temperature recorded at a depth of ½ inch was 63°C., while that at 1 inch was only 53°C.

These results led to a further study in which a portion of a stave from the same churn and about 6 inches long and 2 inches thick was imbedded in plaster of paris in such a way that only one surface (the typical inner surface, as in the churn) was exposed to the water in the water bath. Holes were bored in the stave

parallel to the exposed face and at distances of $\frac{1}{2}$, $\frac{7}{8}$, and $1\frac{3}{8}$ inch from the surface in contact with the water. A few drops of mercury were placed in each hole to insure good contact with the bulbs of thermometers inserted in these holes. Hot water was placed in the water bath and brought to the boiling point as quickly as possible by means of a Bunsen burner placed beneath it. The temperature of the water and wood at the various depths was recorded every five minutes during a period of two hours. Three trials were made. These checked very closely and the results presented in table 1 are averages of the three trials.

As one might expect, there was a lag phase, at the beginning of the exposure, which was more pronounced as the distance from the point of contact increased. In all cases at least 75 per cent of the rise in temperature took place during the first hour. After that, the rise was comparatively slow, and finally the increase was very slight during succeeding intervals. As noted, it required twenty minutes to bring the wood at a depth of $\frac{1}{2}$ inch to the temperature of pasteurization, fifty minutes at $\frac{7}{8}$ inch, and an hour and a half at a depth of $1\frac{3}{8}$ inch, which represents the point at which the staves are jointed. As shown in a previous paper by the authors, since the crevices between churn staves are sometimes infected with mold as far from the surface as these joints, it is obvious that it would be necessary to expose the churn to excessively long treatments with hot water in order to be sure that the molds at such a depth were destroyed.

CHURN TREATMENT STUDIES

a. Treatments with hot water

In the majority of creameries, hot water is, usually, directly available or easily prepared by heating water with steam, and has been most generally used for churn treatment. In the trials reported, the test churn was subjected to several rinsings such as it might receive in an ordinary creamery. Thereafter the treatments were applied.

Trial 1. In this, as in subsequent trials, the churn was revolved during the exposure, for various periods of time. As will

be noted in table 2, the water rinse of the churn previous to exposure to the hot water indicated that the churn was highly contaminated with molds and bacteria. The rinse, obtained after treatment by using sterile skimmilk while the churn was being run for several revolutions, showed that the treatment with water at a temperature ranging from 78.5° to 88°C. was not sufficient to destroy the microörganisms present.

Trials 2, 3, 4, and 5. The amount of water and the periods of exposure were increased in these cases. Otherwise, the same methods used in Trial 1 were repeated. The results obtained were identical, as indicated by the data in table 2.

TABLE 2

Churn treated with hot water

	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4	TRIAL 5	TRIAL 6
Amount of water in churn (gallons)	15	30	30	30	30	30
Fullness of churn	⅛	½	½	½	½	½
Temperature of water at start (°C.)	88	88	85	87	88	96
Temperature of water at finish (°C.)	78.5	80.7	77.1	77.8	73.9	87.0
Period of exposure (minutes)	5	10	20	30	60	30
Churn rinse before treatment (water):						
Molds	+++	+++	+++	+++	+++	+++
Bacteria	+++	+++	+++	+++	+++	+++
Churn rinse after treatment (skim milk):						
Molds	+++	+++	+++	+++	+++	−
Bacteria	+++	+++	+++	+++	+++	+++

Trial 6. In this case, the temperature of the water at the beginning of the period of exposure was brought to 96°C. by introducing steam into the water and bringing the temperature to the desired point before beginning the thirty minute exposure. The effectiveness of this higher temperature is evidenced by the successful results reported in table 2.

Trials 7, 8, and 9. In order to place the method of treatment on a more practical basis, it was decided to continue the hot water treatment on successive days rather than to permit an interval to elapse between exposures during which the molds might develop fruiting bodies. Table 3 gives the data obtained in these trials. The churn in this case was filled with water

which was kept practically at the boiling point during the treatment by means of the introduction of steam at frequent intervals. At the beginning of the trial, the churn was visibly contaminated with mold. The observation on the third day indicated that no conidia were present. The bacteria were in this case somewhat reduced as well. This trial indicated clearly the advantage of regular, daily treatment of the churn.

In order to determine whether or not the improvement was permanent, the churn was allowed to stand idle for four days following these successive treatments, It was rinsed each day with enough cold water to submerge the rollers. After revolving

TABLE 3

Churn treated with hot water on successive days

	TRIAL 7	TRIAL 8	TRIAL 9
Amount of water in churn (gallons)	90	90	90
Fullness of churn	Full	Full	Full
Temperature of water (°C.)	97.7	97.2	97.7
Period of exposure (minutes)	180	180	180
Churn rinse before treatment (water):			
Molds	+++	*	–
Bacteria	+++	*	+++
Churn rinse after treatment (skim milk):			
Molds	–	–	–
Bacteria	++	++	++

* Samples lost.

the churn for several minutes, samples of the rinse water were taken, and the remainder was discarded. It was found that on each succeeding day the evidences of reëstablishment of mold and bacterial growth were more noticeable. Apparently, the mycelia of the molds had not been destroyed by the hot water. The fruiting bodies and free conidia on the surface of the wood were the structures which suffered from the exposure.

b. Treatments with steam

Trial 10. After the churn was given the usual preliminary rinsing with cold water, the steam hose was introduced through the door opening. The door was then closed over it. The steam

was allowed to flow into the churn slowly for a period of three hours. The results given in table 4 clearly show that the treatment was quite successful against the molds. Again, the effect was not permanent, as rinsings taken from the churn four days afterward contained large numbers of molds.

Trials 11, 12, and 13. The same method of steam treatment was applied on three successive days with results as indicated in

TABLE 4

Churn treated with steam

	TRIAL 10
Temperature of steam in churn (°C.)	97.7
Period of exposure (minutes)	180
Churn rinse before treatment (water):	
Molds	+++
Bacteria	+++
Churn rinse after treatment (skim milk):	
Molds	–
Bacteria	+++

TABLE 5

Churn treated with steam on successive days

	TRIAL 11	TRIAL 12	TRIAL 13
Temperature of steam in churn (°C.)	97.7	97.7	97.7
Period of exposure (minutes)	180	180	180
Churn rinse before treatment (water):			
Molds	+	–	–
Bacteria	+++	+++	+++
Churn rinse after treatment (skim milk):			
Molds	–	–	–
Bacteria	+++	+++	++

table 5. As before, the advantage of regular, daily treatment is brought out clearly in this experience. The control of surface growth is very definitely the outcome of an uninterrupted routine of churn treatment.

Following the successive treatments with steam, the churn was again allowed to remain idle and untreated for several days. Observations made daily thereafter showed that the molds quite

soon began normal fructification and the inner churn surface in a short time was spotted with visible areas of mold.

c. Treatments with sodium hypochlorite solutions

Since the use of various chlorine-containing preparations has been quite widely advocated, particularly by commercial concerns, experiments with representative types of these compounds were undertaken.

Trial 14. In the first trial, a solution of sodium hypochlorite containing 54 p.p.m. of available chlorine was used. During the period of exposure, the available chlorine dropped to 35 p.p.m.

TABLE 6

Churn treated with sodium hypochlorite solutions

	TRIAL 14	TRIAL 15	TRIAL 16	TRIAL 17
Amount of solution (gallons)	30	90	30	90
Fullness of churn	¼	Full	¼	Full
Available chlorine in solution at start (p.p.m.)	54	35	200	265
Temperature of solution (°C.)	15.6	10.0	15.6	10
Period of exposure (hours)	½	18	½	18
Churn rinse before treatment (water):				
Molds	++	+++	+++	+++
Bacteria	++	+++	+++	+++
Churn rinse after treatment (skim milk):				
Molds	++	+++	+++	+++
Bacteria	++	+++	+++	+++

The results given in table 6 show that the solution had no noticeable effect upon the molds or bacteria.

Trial 15. Using the same methods, but with a larger amount of solution, somewhat lower in available chlorine, and over a longer period of time, the results obtained were negative as shown in table 6.

Trials 16 and 17. Inasmuch as solutions with the concentration of available chlorine commonly recommended did not give successful results, much stronger solutions were used. The results were negative.

d. Treatments with solutions of alkaline, crystalline hypochlorite

Trials 18, 19, 20, and 21. A type of hypochlorite in crystalline

form in combination with tri-sodium phosphate was used in solutions of various strengths. Concentrations considered as ordinary and high were used during long and short exposures. The results given in table 7 indicate definitely that the solutions were

TABLE 7

Churn treated with solutions of alkaline, crystalline hypochlorite

	TRIAL 18	TRIAL 19	TRIAL 20	TRIAL 21
Amount of solution (gallons)	60	90	60	90
Fullness of churn	⅔	Full	⅔	Full
Available chlorine in solution at start (p.p.m.)	62	53	283	274
Temperature of solution (°C.)	10	13.3	15.6	12.2
Period of exposure (hours)	2	18	2	18
Churn rinse before treatment (water):				
Molds	+++	+++	+++	+++
Bacteria	+++	+++	+++	+++
Churn rinse after treatment (skim milk):				
Molds	+++	+++	+++	++
Bacteria	+++	+++	+++	+++

TABLE 8

Churn treated with chloramine-T solutions

	TRIAL 22	TRIAL 23	TRIAL 24	TRIAL 25
Amount of solution (gallons)	30	50	90	90
Fullness of churn	⅓	⅔	Full	Full
Available chlorine in solution at start (p.p.m.)	53	46	300+	350
Temperature of solution (°C.)	51.6	51.6	48.8	48.8
Period of exposure (hours)	2	18	2	18
Churn rinse before treatment (water):				
Molds	+++	+++	+++	+++
Bacteria	++	+++	+++	+++
Churn rinse after treatment (skim milk):				
Molds	+++	++	+++	+++
Bacteria	+++	+++	+++	+++

not effective in destroying the surface molds in the churn. As in the case of ordinary sodium hypochlorite solutions, there was a noticeable decrease in available chlorine during the time the solutions were in the churn.

e. Treatments with chloramine-T solutions

Trials 22, 23, 24, and 25. A more stable chlorine-bearing compound, a chloramine-T was employed, in these trials in solutions of various concentrations and for different periods of time. None of these trials gave satisfactory results as will be noted in table 8, although there was a very slight improvement in the conditions in the churn in Trial 23. The strength of the solution diminished during the longer periods of exposures. Altogether, the chloramine solutions used were ineffective.

DISCUSSION

It has been established beyond all doubt that churns do become infected with various microörganisms. The problem then resolves itself into a search for the most effective methods for reducing this infection to a minimum.

The results presented in the preceding pages indicate very definitely that of the methods used, sufficient exposures to water at sufficiently high temperatures and freely flowing steam were the most satisfactory.

In the preliminary studies on the heat penetration of wood, it was evident that it required considerable time for the temperature of the wood to be raised to a point sufficiently high to be effective in destroying even vegetative forms of microörganisms. Since the infected areas may be at some distance from the inner surface of the churn, it is obvious that this factor must be taken into account if all infection is to be overcome. Under practical creamery conditions, it might be considered too costly and too laborious a task to use sufficiently large quantities of hot water. Such extreme exposures might have an undesirable effect upon the churn itself. A possible modification of prolonged treatment is suggested as the result of the trials. Where moderate quantities of water, sufficient to cover the rolls, are used at high temperatures and for reasonable periods of time, comparatively satisfactory results are obtained if such treatment is repeated daily without interruption. Under these conditions, the surface of the wood at least is kept in a good sanitary condition, and fructification from concealed mycelia of molds especially is

inhibited. With the churn in daily use and with the wood thoroughly swollen so that the normal expansion tends to keep the crevices between staves, etc., more nearly closed, the daily treatment with hot water gives reasonably satisfactory results. This daily treatment cannot be over-emphasized.

In the case of steam, satisfactory results were obtained, especially when the treatment was applied without fail each day. With steam, however, the effects upon the churn, especially the blistering of the paint, may be sufficient to discourage its use.

The use of chlorine-bearing solutions gave negative results consistently. Even with high concentrations of available chlorine, the effect upon the microflora of the churn was scarcely noticeable. In the experimental churn used in these tests, conditions under which chlorine compounds had to work were not nearly as bad as they usually are in regularly used commercial churns, where the presence of a film of butter fat makes it more difficult for the solution to reach the microörganisms. Based upon the results obtained in these studies, solutions of sodium hypochlorites, crystalline alkaline hypochlorites, or chloramines could not be recommended. In the case of the alkaline washing solutions in general, there is an undesirable effect upon the wood which discourages their use. With these alkaline solutions there appears to be a softening of the wood surface which leads to difficulty.

The fact that treated churns when allowed to stand idle for several days become a serious source of infection for cream must not be overlooked. Regardless of the apparent effectiveness of any method of treatment as measured by the examination of skimmilk used as a rinse immediately after treatment, the organisms were not completely eliminated, as studies made several days later showed the churn to be very much infected. Treatment merely improved the conditions but did not remove the cause.

The molds were more readily destroyed than the bacteria. In fact, in no instance were the bacteria eliminated. Apparently, heat- and chlorine-resistant forms established themselves in the churn and resisted all efforts to overcome them. Hot water and steam were most effective against them.

In general, the results obtained in these preliminary studies

with a highly infected churn demonstrate that the most satisfactory method of treatment used was water at a high temperature for a sufficient period of time and repeated daily without interruption. Further investigations are to be made using other methods and expanding the work to include trials on churns in daily use in commercial creameries.

SUMMARY

1. Various methods for treating a churn to rid it from micro-organisms were investigated.

2. When the churn was partially filled with water at 96°C. and revolved for one-half hour, the surface molds were destroyed.

3. The hot water treatment was most effective when carried out daily.

4. Failure to treat the churn daily resulted in the reappearance of mold on the inner surface of the churn.

5. The use of steam for sufficient periods of time was effective against the molds but caused the paint on the churn to blister badly.

6. Solutions of sodium hypochlorite, alkaline crystalline hypochlorite, and chloramine-T were ineffective.

7. None of the treatments was especially effective against bacteria.

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THE SOLUBILITY OF METALS IN MILK

I. THE SOLUBILITY OF COPPER UNDER VARIOUS CONDITIONS*

H. T. GEBHARDT AND H. H. SOMMER

The harmful effect of dissolved copper in dairy products on their flavor and keeping quality is now quite generally recognized. Experimental results and practical experience have demonstrated that dissolved copper is capable of producing a "cardboard" or "cappy" flavor in fresh milk and cream, and that dissolved copper is an important factor in the development of tallowy and, in some cases, fishy flavors in such products as butter, condensed milk, frozen cream, and whole milk powder. The solubility of copper in dairy products under various conditions is therefore of direct practical importance.

Equipment constructed of copper, copper alloys and tinned copper is in extensive use in the dairy industry. This is true because of installations that were made before the deleterious effect of copper was recognized as it is at present, and because of new installations in which such metals are used on account of relative cheapness, mechanical advantages in the working of the metals and high heat conductivity. Such use of copper equipment may not be disastrous in all cases because the rate of copper solution is variable. Accurate information is therefore desirable concerning the factors that affect the rate of copper solution so that the conditions may be recognized under which copper equipment may or may not be used.

DISCUSSION OF PREVIOUS WORK

The solubility of copper in milk has been studied by various investigators (1, 5, 6, 7, 8, ,9, 11, 12). These studies have revealed many data and interesting facts on copper solubility in

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milk, but unfortunately the value of this information is greatly reduced (*a*) the data of the various investigators are not comparable, and (*b*) their experimental methods are more or less open to criticism.

The most comprehensive studies have been carried out by Rice and Miscall (12), and later by Quam et al. (11) and by Miscall et al. (7). However, the data on the amounts of copper dissolving in milk under certain conditions do not agree or are not comparable. Quam et al., reported the temperature of maximum solubility of copper in milk as 85°C. in 1928 (11), and as 90°C. in 1929 (9), while Miscall et al. (7) reported it as 66°C. in raw milk 60°C. in pasteurized milk. Quam et al. exposed the copper to the milk for thirty minutes, and Miscall et al., for one-hundred and twenty minutes. Quam attributed the lower temperature of maximum solubility as reported by Miscall et al., to chemical changes that occur in the milk during the longer exposure.

The method used by Rice and Miscall (12) and by Miscall et al. (7) for exposing the copper to milk was to place 14 twisted strips of copper, 1 by 5 inches, into 2 liters of milk in a 5-liter flask, for two hours without agitation. It is known that the relative velocity of the liquid to the metal is an important factor in metal corrosion; Frazer et al. (3) found that the rate of corrosion increases with the velocity and approaches constancy when a velocity of 200 feet per minute is attained. In view of this, the lack of mechanical agitation in the above investigations must be considered a serious fault. The only agitation was that resulting from convection currents, and as such must have varied with the temperature and other conditions. These considerations limit the value of the investigations cited above, and the data obtained are comparable only with data obtained by a similar procedure.

The method used by Quam et al. (11) for exposing the copper to milk, provided an alternating flow of the milk over the crimped metal test pieces. A higher relative velocity of liquid to metal might have been desirable; but the main criticism of this procedure is that the aeration of the exposed film of milk (*a*) affected the gaseous content so that the results cannot be considered comparable to submerged corrosion tests, and (*b*) provided op-

portunity for evaporation so that the temperature of the exposed film was undoubtedly lowered, especially in the tests conducted at higher temperatures. These criticisms are especially applicable in view of the corrosion factors as enumerated by Frazer et al. (3), which emphasize that in corrosion tests the important factors are the composition of the liquid including dissolved gases, the relative velocity of the liquid to the metal, and the temperature.

Rice and Miscall (12) have reported that previously boiled milk dissolves less copper than raw milk, while Miscall et al. (7) have reported that pasteurized milk dissolves more copper than raw milk. No explanation is offered by the above investigators for these phenomena, but the suggestion is made that these are probably related to chemical changes produced in the milk by the heating.

These phenomena are undoubtedly related to the reduced solubility of copper in milk at temperatures beyond that of maximum solubility. Quam (9), citing Speller (13), suggested that the break in the temperature-solubility curve of copper in milk is due to the decreased solubility of oxygen in milk at higher temperatures. This suggested explanation can hardly be adequate, because there is no such break in the solubility curve of oxygen in water as given by Speller (13).

PURPOSE OF THE PRESENT STUDY

The present experimental work was undertaken (*a*) to review the findings of previous investigators, (*b*) to accumulate additional data on the solubility of copper in milk under various conditions, (*c*) to seek the causes of the reduced solubility of copper in milk at temperatures above the point of maximum solubility, and the changed solubility in preheated milk, and (*d*) to gain a better theoretical understanding of the processes involved in the solution of copper in milk.

EXPERIMENTAL METHOD USED

a. Apparatus and methods for corrosion tests

The method for exposing the copper to the milk was designed to give staisfactory control of (*a*) the composition of the milk,

(especially gaseous content), (*b*) the relative velocity of the test pieces to the milk, and (*c*) the temperature of the milk. This includes all the fundamental factors which are known to determine the usefulness of laboratory corrosion tests (Frazer et al. (3).

The metal test pieces were either thin sheets of copper 1 by 2 inches (these were used in all of the first experiments) of 25.8 cm.^{2} surface, or else, samples of commercial sheet copper 1 by 3 inches, $\frac{1}{16}$ inch thick, of 39.66 cm.^{2} total surface.

These test pieces were exposed to 500 cc. milk,[1] contained in 1 quart milk bottles *1*. (See diagram, *fig. 1.*) A stirring device passing through rubber stopper *2* entered the neck of the bottle. The stirring device consisted of supporting arm *3;* bearing *4*, shaft *5*, chuck *6*, collars *7*, fly wheel *8*, and attachment *9*, of the flexible cable *10* to the shaft. A glass stirring rod *11*, was held firmly by chuck *6;* the glass rod *11* was provided with small side-arms of glass (upper arms *12*), and over the lower end of the rod fitted the glass tube *13* which was also provided with side-arms of glass (lower arms *14*). This lower tube was held in position by the upward pull of the rubber bands *15*, suspended from the hooks *16* on the chuck. The metal test pieces *17* were held in small grooves of the side arms between the upper and lower side arms by the tension of the rubber bands *15*. Cup *18* was provided to collect any condensate water flowing down from the upper metal parts of the bearing and the chuck. Through glass tubes *19*, gases of the desired nature could be passed through the milk. There was a hole in the side of the bottle to permit the entrance of the thermometer *20*, an electrode *21*, and a salt bridge *22* (saturated KCl agar) through the stopper *23*.

The closure at *2* was airtight and did not permit any exchange of gases because the bearing was 3 inches long, closely fitting, and was kept well oiled.

Four bottles of this type were prepared. All four stirring shafts were driven through flexible steel shafts by an electric motor. The speed of the motor was reduced, and the drive thus obtained was at the rate of 210 r.p.m. for all four shafts.

[1] In certain cases 530 cc. milk was used.

These bottles were placed either in separate water-baths or else, as in the later experiments, all were placed in the same water-bath. The water-baths were heated with gas, with the heating flame regulated by means of a toluol—mercury thermo-regulator, which kept the temperature constant within 1°. Deviations from this rule will be reported in connection with the presentation of the experimental results.

In the experiments where the thin copper sheets were used, these were fastened by pinching them between a 2 mm. glass rod and the stirring rod, which were held together by the tension of small rubber bands. In this manner practically the entire 25.8 cm.^{2} of copper surface was exposed, only a narrow line between the two glass rods being covered. Although the number of revolutions was constant, the relative velocity was not uniform at various places over the copper surface; it was higher at the periferal portion, and lower towards the center. These conditions, however, were quite uniform as indicated by the uniformity of the results obtained.

These conditions were improved in the method of attachment used for the larger copper blades. These were held rigidly in a vertical position parallel to the stirring rod and about 1 inch from the center. This arrangement facilitated the exchange of the test pieces. Both sides of the copper blades were exposed at nearly the same velocity, and only a relatively small area of the metal was unexposed, viz. the small area at the edges in contact with the holding arms.

This arrangement provided as nearly ideal conditions of uniform velocity as can be expected. Absolute uniformity of velocity over the entire surface would necessitate test pieces to fit perfectly the stream lines in the liquid. It appeared practically impossible to avoid entirely any unexposed areas at the points of attachment. Evans (2) proposed a method for accomplishing this; but because of the chance of slight losses due to abrasion of the metal, and for various practical reasons, the method of Evans was not followed.

In the present arrangement all the controllable variables as described by Frazer et al. (3) have received consideration. A

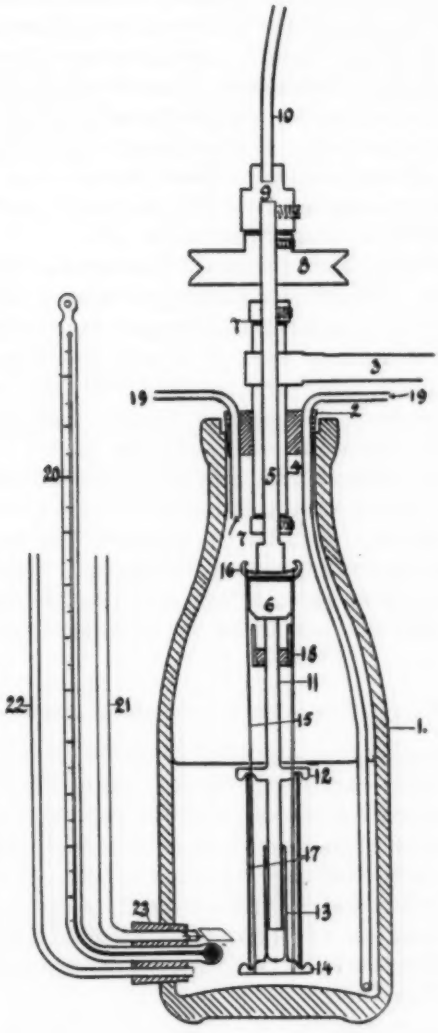

FIG. 1

definite and sufficiently large quantity of the corrosive liquid was provided. The temperature of the water bath and that of the milk was controlled. Further, this arrangement permitted the bubbling of gases of the desired nature through the milk, practically the same amount of gas passing through all four bottles when connected in series. It is recognized that a higher velocity (revolutions per minute) might have been chosen to good advantage and that some device might have been provided to prevent the continuous circular flow of the milk.

It is known that the condition of the metal surface influences metal solution. Therefore, an attempt was made to prepare the metal surfaces so as to furnish the same type of surface in every test. This was done for the 1 by 2 inch thin copper sheets by cleaning in dilute nitric acid, or later by short immersion in bichromate-sulphuric acid cleaning mixture, followed by rinsing in water. The copper sheets were then placed in fresh milk for five to ten minutes, until they were used for the test. The larger copper blades were treated in a different manner. The method chosen was intended to simulate the nature of well cleaned metal surfaces in dairy equipment. The test pieces were cleaned by scouring with "Bon Ami," just enough to remove any tarnish, but care was taken not to bring about a polishing effect.

b. Method for determining the amount of copper dissolved

The amount of copper dissolved was determined in two different ways. In the earlier experiments, when thin copper sheets were used, the milk was ashed and the copper in it was determined by analysis using the method as already published (4). These results were originally expressed in terms of milligrams of copper dissolved in 1 liter of milk after exposure to the 25.8 cm.^{2} of copper surface for two hours. Later these results were expressed as milligrams of copper dissolved per square decimeter of copper surface per hour.

In the later experiments the losses were determined directly from the difference in weight of the test pieces before and after exposure. The metal test pieces after cleaning in the manner

as described above were prepared for weighing by rinsing in alcohol and finally in ethyl ether. Before they were weighed, they were kept in a desiccator at room temperature for at least one hour. The weighed test pieces were then placed between the holders of the stirring device, and subjected to the test. After exposure they were quickly removed, and rinsed in cold tap water. Any adhering milk constituents were removed by means of a cotton swab. The blades were then rinsed in distilled water, blotted dry, rinsed in alcohol, and finally in ether. Before weighing they were again kept in a desiccator for at least one hour. The results were finally expressed in terms of milligrams loss per square decimeter, per hour.

EXPERIMENTAL RESULTS

1. The effect of acidity on the solubility of copper in milk

It is generally assumed that the acidity of the milk is an important factor in the solution of metals by milk. The study of the effect of hydrogen-ion concentration was therefore given first consideration. In the experiments that were conducted on the effect of acidity, this factor was varied and the temperature and other factors were kept constant. The experimental details and the results are presented in table 1. A study of the data leads to the following conclusions:

1. Small variations in acidity produced no perceptible effect on the solubility of copper in milk.
2. Higher acidities reduced the solubility of copper.
3. Neutralizing the milk to an alkaline reaction did not perceptibly influence the solubility of copper.
4. The effect of acidity is the same whether it is produced by the addition of pure lactic acid or by biological agencies.

The experiments were primarily intended for comparison within each series only; the samples within each series were taken from the same lot of milk. A comparison between the series, however, is possible and quite instructive. Samples *a* to *d*, which were skim milk, when compared with all the other samples, which were whole milk, indicate that copper is less soluble in skim milk

than in whole milk. Other factors may have been involved such as differences in the gaseous content occasioned by differences in the age and previous handling of the milk. However, it should be noted here than all the milk samples were taken from the Uni-

TABLE 1

The effect of acidity on the solubility of copper in milk

SAMPLE	pH	TITRATABLE ACIDITY; LACTIC ACID	ACIDITY PRODUCED BY:	TEMPERATURE	COPPER CONTENT IN MGM./L.	COPPER DISSOLVED IN MGM./DM.²/HR.*
		per cent		°C.		
a	6.54	0.17–0.16	Nothing added	20	0.69	0.55
b	6.54	0.17–0.16	Nothing added	20	0.62	0.49
c	5.72	0.33–0.32	Dilute lactic acid	20	0.69	0.55
d	5.72	0.33–0.32	Same as c	20	0.62	0.48
e	4.58		19.2 cc. N/1 lactic acid	19.5	1.14	0.97
f	5.00		15.0 cc. N/1 lactic acid	19.5	1.02	0.86
g	5.30		6.6 cc. N/1 lactic acid	19.5	1.55	1.37
h	6.30		Nothing added	19.5	1.43	1.32
i	6.56		Nothing added	20	1.28	1.06
j	3.28		Dilute lactic acid	20	0.60	0.41
k	3.28		Same as j	20	0.69	0.49
l	8.42		Dilute NaOH	20	1.23	1.01
m			Pasteurized milk, nothing added	20		0.90
n	4.03		Ripened 24 hours	20		0.82

Experimental details:

Surface of copper: 25.8 cm.² for samples a–l; 39.7 cm.² for samples m and n.

Time of exposure: Two hours for samples a–l; one hour for samples m and n.

Amount and kind of milk used: 500 cc. skimmilk for samples a–d; 500 cc. whole milk for samples e–l; about 500 cc. whole milk for samples m and n.

* Dissolved copper was calculated based on the determined copper content of the original milk in samples e–h, (0.136 mgm. per liter), and in samples i–l, (0.182 mgm. per liter), or calculated by assuming that the copper content of the original milk was 0.12 mgm. per liter for samples a–d, and 0.15 mgm. per liter for samples m and n.

versity herd milk in which the bacterial count was known to be well below 10,000 per cubic centimeter, and the samples were handled in such a manner as to exclude secondary effects due to bacterial growth.

2. The effect of dissolved gases on the solubility of copper in milk

It has been demonstrated by various investigators that the dissolved oxygen in the corrosive liquid plays an important rôle in metal solution. Thus it is apparent that the gaseous constituents of milk, which may be ignored in many dairy manufacturing problems, are likely to be of utmost importance here. With this in mind experiments were undertaken to determine the effect of evacuation, aeration, and of treatment with oxygen, and carbon dioxide, on the solubility of copper in milk. The results and experimental details are given in table 2. The following conclusions may be drawn from the results obtained:

TABLE 2

The influence of dissolved gases on the solubility of copper

SAMPLE	TREATMENT	TEMPERATURE	COPPER CONTENT OF MILK IN MGM./L.	COPPER DISSOLVED IN MGM./DM.^{2}/HR.*
		°C.		
a	None	Room	0.493	0.37
b	Vacuum, 4 hours	Room	0.440	0.31
c	Air	Room	0.834	0.66
d	None	Room	0.562	0.40
e	None	20.5	0.683	0.52
f	None	20.5	0.714	0.55
g	Carbon dioxide	26.0	0.317	0.16
h	Oxygen	26.0	1.645	1.45
i	Oxygen	23.0	1.580	1.38
j	Oxygen	62.8	12.240	11.70
k	Oxygen	62.8	(Direct gravimetric)	17.65

Experimental details:

Surface of copper: 25.8 cm.^{2}, except in sample k, where it was 39.7 cm.^{2}

Time of exposure: Two hours, except in sample k, where it was one hour.

Amount of milk used: 500 cc. skimmilk was used in samples a and b, fresh whole milk in all other samples.

* For calculating the dissolved copper, the original copper content of the milk was assumed to be 0.15 mgm. per liter for whole milk, and 0.12 mgm. for skimmilk.

1. Evacuation of the milk previous to exposure to copper slightly decreases the amount of copper dissolved.

2. The bubbling of air through the milk during the exposure to copper increased the amount of copper dissolved very slightly.

3. The bubbling of carbon dioxide through the milk during the exposure to copper very markedly decreased the amount of copper dissolved.

4. The bubbling of oxygen through the milk during exposure to copper appreciably increased the amount of copper dissolved. The effect was more pronounced at pasteurizing temperature than at room temperature.

3. The effect of other milk constituents on the solubility of copper in milk

No experimental study was made of the effect of other milk constituents on copper solubility, although they are undoubtedly involved in the corrosive action. However, the individual milk constituents, in all probability, do not exert their effects independently, and therefore any attempt to study the isolated constituents singly would lead to erroneous conclusions. If a study of the effect of individual milk constituents were to be undertaken, it would be necessary to vary the amount of one in the presence of constant amounts of all the other constituents. In view of the immense amount of work that would be required in preparing the samples and in studying this phase of the subject adequately, in view of the fact that the milk constituents in mixed milk are reasonably constant (certainly as far as corrosive effect may be concerned), and in view of the fact that the results could not lead to any practical control of the problem, no work of this nature was undertaken. Nevertheless it is of interest to speculate on the rôle of the various milk constituents in metal solution.

The effect of the organic constituents appears to be of an indirect nature. Lactose is a reducing sugar and as such might be expected to exert an effect on copper solution. However, its reducing effect is pronounced only in alkaline solutions, and therefore probably plays no important rôle in the solution of copper by milk.

The milk fat cannot take part directly in any ionic process such as metal solution, yet there are indications that whole milk has a greater solvent action on copper than skim milk (see table 1, compare samples *a* to *d* with *e* to *n*). More data are needed

before a definite conclusion may be drawn concerning the effect of fat in milk on copper solution.

The protein substances in the milk are only slightly ionized, and would therefore appear to be indifferent in the process of metal solution. However, insoluble protein salts of the heavy metals may be formed, thereby promptly removing the dissolved metal from solution. As a result proteins are likely to affect metal solution indirectly.

In this connection it is of interest to recall that the colloidal constituents of milk bear electric charges which bring about the migration of these particles under the influence of an external electromotive force. Metal salts may influence the electric charges sufficiently to alter the properties of the milk as a colloidal suspension or emulsion. These phenomena are probably related to the difference in the behavior of metal surfaces with respect to the baking on of the milk solids.

The ionic constituents of the milk, viz. the salts, appear to be most directly involved in the solution of metals in milk, because the salts comprise the anions which may combine with the metal, and determine the hydrogen-ion concentration. From later studies, however, it appears that the salts are not the most important factors in metal solution.

4. The effect of temperature on the solubility of copper in milk

The velocity of chemical reactions increases with the temperature. For this reason the rate of copper solution in milk is expected to increase with the temperature. Previous investigators have shown that this expected effect is realized only up to a certain temperature, beyond which there is actually a decrease in the amount of copper dissolved. However, there is a lack of agreement concerning the temperature of maximum solubility. Further, no explanation, founded on adequate experimental evidence, has been offered to account for the decreased solubility of copper at temperatures beyond that of maximum solubility. Experimental results on this subject are therefore of special interest both from a theoretical and practical standpoint.

The experimental results on the solubility of copper in milk at various temperatures during exposures of thirty, sixty, and one

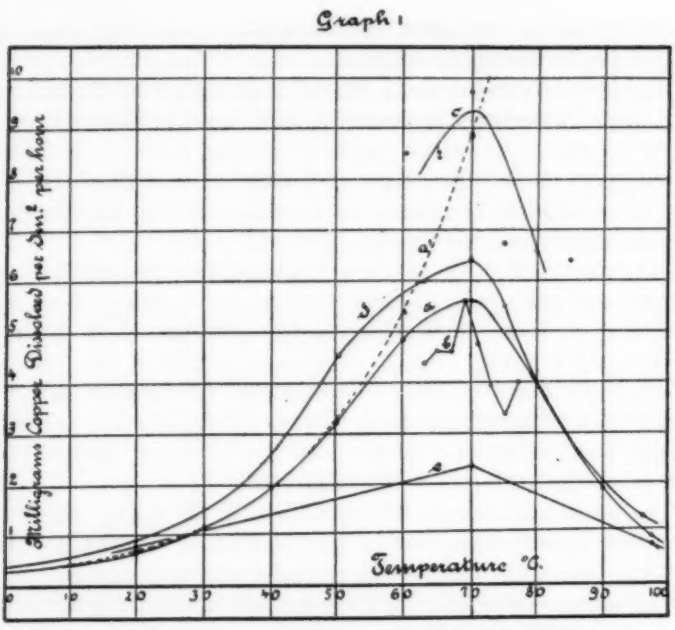

FIG. 2. *a*. Curve showing copper solubility in mgm./dm.²/hr., calculated from gravimetric determinations of the dissolved copper in milk after two hours exposure to 25.8 cm.² surface of copper at temperature intervals of 10°C.

a' Curve showing copper solubility in mgm./dm.²/hr., calculated from the formula:

$$\text{Log } R = a + b \cdot t^\circ; \text{ Log R} = 1.414 + 0.02195\ t^\circ$$

where R = mgm. Cu/dm.²/hr. a and b—calculated from the experimental data at 10° and at 40°C.

b. Same as *a*,—but at 2° temperature intervals from 63° to 77°C.

c. Same as *a*,—but thirty minutes exposure only.

d. Curve showing copper solubility in mgm./dm.²/hr., calculated from the losses in weight of strips of plate copper of 39.7 cm.² surface, exposed to milk for one hour at 20°, 60°, 62.8°, 70°, 75°, 85°, and 97°, average of 4 to 8 determinations.

e. Same as *d*,—but in acid milk at 20°, 70°, and 97°C.

hundred and twenty minutes, are presented in figure 2. A study of this figure and of the data on which it is based leads to the following conclusions:

1. Temperature is the most important of the factors that determine the amount of copper dissolving in milk. Up to a temperature of 50°C. the solubility increases at a logarithmic rate as expressed in the temperature-reaction velocity equation:

$$\log R = a + b.\, t^{\circ}$$

2. The temperature of maximum solubility is 70°C.; this holds true whether the time of exposure is thirty minutes, one hour, or two hours. This finding is contradictory to the contention of Quam, that upon exposures longer than thirty minutes, chemical changes occur in the milk which change the temperature of maximum solubility.

3. The rate of copper solution becomes less as the time of exposure is increased.

4. The decrease in the solubility of copper in milk at higher acidity, as observed at room temperature (see table 1), is much more marked at higher temperatures.

It should be noted that the above conclusion refers to the solubility of copper and not copper oxide, as on a tarnished surface. The good results obtained by the use of hot, sour whey for cleaning tarnished copper equipment are not to be explained by the specific action of sour whey on copper, but rather on the copper oxide which apparently is dissolved quite readily by sour whey. However, sweet milk also dissolves films of copper oxide quite readily.

5. The effect of the previous heat treatment of milk on copper solubility

Rice and Miscall (12) have reported not only that less copper dissolved in milk at boiling temperature than at room temperature, but also that milk, after it had been boiled and cooled, dissolved less copper than raw milk. Raw milk dissolved 16.25 mgm. copper per liter at room temperature, while milk previously boiled and cooled at room temperature dissolved 5.35 mgm. copper per liter, and saturation with air in the latter case increased the amount only to 5.45 mgm. This enormous difference in the solubility of copper apparently was related to some change in the composition of the milk. A study of these phe-

nomena promised to elucidate some of the fundamental factors that influence the solubility of copper in milk.

The findings of Rice and Miscall were partially confirmed by the results of the experiment presented in table 3, but the differences found between the solubilities in raw and previously boiled milk were slight at room temperature, and even at boiling temperature, they were not so great as reported by these investiga-

TABLE 3

The solubility of copper in preheated milk

SAMPLE	HEAT TREATMENT OF MILK	TEMPERATURE	COPPER CONTENT OF MILK IN MGM./L.	COPPER DISSOLVED IN MGM./DM.²/HR.‡
		°C.		
a	Raw milk	Room	0.737	0.60
b	Raw milk	Boiling	2.535	2.34
c	Previously boiled*	Room	0.615	0.48
d	Previously boiled	Boiling	1.561	1.40
e	Raw milk	Room	0.515	0.38
f	Raw milk	62.8	3.945	3.68
g	Previously pasteurized†	Room	0.845	0.68
h	Previously pasteurized	62.8	4.005	3.83

Experimental details:

Surface of copper: 25.8 cm.²

Time of exposure: Two hours.

Amount of milk used: 500 cc. (skimmilk).

* The milk was heated to about 97°C. in a boiling water bath for about twenty minutes, then cooled.

† The milk was previously pasteurized by heating to 62.8°C. for thirty minutes, then cooled.

‡ Calculated on the assumption that the copper content of the original skimmilk was 0.12 mgm. per liter.

tors. Their results are probably to be attributed to the lack of agitation in their experimental procedure. An experiment of a similar nature (table 3, samples *e* to *h*) showed that previous pasteurization did not decrease copper solubility, but rather increased it slightly.

Knowing of the important rôle of oxygen in copper solution, it was decided to conduct preliminary experiments seeking the explanation for the above phenomena in the dissolved oxygen.

The previously boiled milk was saturated with oxygen by bubbling oxygen through the milk during the entire exposure to copper, and the amount of copper dissolved was compared with that dissolved by raw milk similarly treated. The results, given in table 4, samples *a* to *d*, were unexpected; even when saturated with oxygen, the previously boiled milk dissolved less copper than raw milk. Similar results (samples *e* to *f*) were obtained when air was used instead of oxygen.

TABLE 4

The effect of dissolved gases on the copper solubility in preheated milk

SAMPLE	TYPE OF MILK	TREATMENT OF MILK	TEMPERATURE	COPPER CONTENT OF MILK			COPPER DISSOLVED IN MGM./DM.²/HR.*
				Determination		Mean	
				1	2		
			°C.	mgm./l.	mgm./l.	mgm./l.	
a	Raw	Oxygen	23.0	1.658	1.499	1.597	1.38
b	Raw	Oxygen	62.8	11.630	12.850	12.090	11.70
c	Previously boiled	Oxygen	23.0	1.051	1.164	1.108	0.93
d	Previously boiled	Oxygen	62.8	9.970	9.490	9.760	9.30
e	Raw	Air	28.5	0.307	0.302	0.304	0.15
f	Raw	Air	62.8	3.940	3.980	3.960	3.69
g	Previously boiled	Air	28.5	0.403	0.365	0.384	0.23
h	Previously boiled	Air	62.8	2.870	2.810	2.840	2.58

Experimental details:
Surface of copper: 25.8 cm.²
Time of exposure: two hours.
Amount of milk used: 500 cc.
Amount of milk ashed: 250 cc.

* Calculated, assuming that the copper content of the original milk was 0.15 mgm. per liter.

Other experiments of a similar nature were then conducted but using preheating temperatures of 60, 70 80 and 90°C. for one hour. The heating was done in the quart milk bottles which were to be used later for the solubility tests. During the preheating the milk was agitated by means of the same device used in the solubility tests but with glass slides in place of metal test pieces. After the heating and holding at the desired temperature for one hour, the milk was promptly cooled to the desired

temperature by running cold water into the water bath. The results of the various experiments are given in table 5.

In the first series of samples, the milk was cooled to 60°C. and held at that temperature for ten minutes, the copper test pieces

TABLE 5

The solubility of copper in preheated milk

SERIES	SAMPLE	HEAT TREATMENT	TREATMENT DURING EXPOSURE	TEMPERATURE	COPPER CONTENT OF MILK IN MGM./L.	COPPER DISSOLVED IN MGM./DM.²/HR.
		°C.		°C.		
1	a	60 (1 hour)	None	60	4.340	4.05
	b	70 (1 hour)	None	60	4.868	4.56
	c	80 (1 hour)	None	60	2.750	2.52
	d	90 (1 hour)	None	60	3.555	3.30
2	e	60 (1 hour)	Bubbling oxygen	23.5	1.841	1.64
	f	70 (1 hour)	Bubbling oxygen	23.5	2.445	2.22
	g	80 (1 hour)	Bubbling oxygen	23.5	1.937	1.73
	h	90 (1 hour)	Bubbling oxygen	23.5	2.095	1.88
3	i	90 (2 minutes)	Bubbling oxygen	24.5	1.928	1.72
	j	90 (20 minutes)	Bubbling oxygen	24.5	1.959	1.75
	k	90 (40 minutes)	Bubbling oxygen	24.5	2.252	2.04
	l	90 (60 minutes)	Bubbling oxygen	24.5	1.944	1.74
4	m	60 (1 hour)	Carbon dioxide	60	0.874	0.70
	n	70 (1 hour)	Carbon dioxide	60	0.701	0.53
	o	80 (1 hour)	Carbon dioxide	60	0.535	0.37
	p	90 (1 hour)	Carbon dioxide	60	0.419	0.26

Experimental details:
Surface of copper: 25.8 cm.²
Time of exposure: Two hours.
Amount of milk used: 500 cc. (whole milk).
Calculated on the assumption that the copper content of the original milk was 0.15 mgm. per liter. The milk used in each experimental set was taken from the same lot of milk, so that the results are not influenced by variations in the composition of the milk.

were then substituted for the glass slides, and agitation continued for two hours at 60°C. The results again show a reduced copper solubility when the milk had been preheated to temperatures above 70°C. It will be noted in this series of samples as in the following series, that milk preheated at 70°C. for one hour

dissolved more copper than milk preheated at 60°C. These results appear to parallel the increased copper solubility in pasteurized milk, observed by Miscall et al. (7).

In the second series of samples, the milk was cooled to 23.5°C. and held at that temperature for ten minutes; the copper test pieces were then substituted for the glass slices and the bubbling of oxygen through the milk was started; agitation, the bubbling of oxygen and exposure to copper were then continued for two hours at that temperature. The results (samples *e* to *f*) indicate that the bubbling of oxygen through the milk tends to eliminate the effect of preheating on the amount of copper dissolved.

In the third series of samples all the milk was preheated to 90°C. using the same procedure as before, but varying the holding time. After the holding time had elapsed, the samples were cooled to 24.5°C. and exposed to copper under the same conditions as in the second series. The results (sample *i* to *l*) lead to a similar conclusion as in the preceding series.

In the fourth series of samples the procedure was the same as in the first series, but bubbling of carbon dioxide through the milk was started when the copper test pieces were placed in the milk. The results (samples *m* to *p*) indicate that the expulsion of dissolved oxygen apparently is not the only effect of the heat treatment. However, this indication is not conclusive, because not sufficient time was allowed for the complete removal of the oxygen by rinsing with carbon dioxide, before the milk was exposed to the copper.

These preliminary experiments indicate that the effect of preheating on copper solubility must be sought in the gaseous content of the milk. A comparison of samples *m* to *p* with samples *a* to *d* demonstrates the effects of the removal of dissolved oxygen on copper solubility. It should be noted that even at 60°C. the copper solubility in samples *m* to *p* is less than would normally be expected at room temperature.

If the dissolved gases affect copper solubility, they probably exert this effect through their influence on the oxidation intensity which can be measured in terms of oxidation-reduction potentials. It was therefore decided to undertake experiments at-

tempting to correlate oxidation-reduction potentials of the milk under various conditions to copper solubility.

6. The solubility of copper in relation to the oxidation-reduction potential

The solutions of metals, however complicated the mechanism of solution may be, fundamentally involves the change of the reduced form of the metal to an oxidized form, or a change of the free energy of the metal. Oxidation may be produced by chemical agents, or by electrochemical means. The effect of atmospheric oxygen in metal solution is commonly appreciated, but in addition the literature on metal corrosion gives reports of agents other than oxygen accelerating metal solution, such as ferric ions, cupric ions and chlorine.

The conception of metal solution as an oxidation process is not new, but few attempts have been made to correlate the rapidity of metal corrosion to the oxidative power of corrosion media, and no attempts have been made to correlate corrosion to oxidation-reduction potentials.

THE PROCEDURE USED IN DETERMINING THE OXIDATION-REDUCTION POTENTIALS

For the measurement of the oxidation-reduction potentials, freshly gold plated platinum electrodes were used. The milk was connected to a M/10 calomel half-cell by means of a saturated potassium chloride-agar bridge. The potential between the gold plated platinum electrode and the terminal of the calomel half-cell was measured by means of a Leeds Northrup, type K, potentiometer. The temperature of the calomel cell was constant within 24° to 26°C., and in each case the temperature was noted and proper corrections applied in the calculation of the E_h.

By means of the oxidation-reduction potential measurements it was hoped to find whether the peculiar temperature-solubility curve for copper in milk could be explained in terms of oxidation intensity. An attempt to measure the oxidation-reduction potential in milk at varying temperatures up to boiling temperature, may at first appear erroneous, because of the wide

difference in temperature between the unknown and the calomel half-cell. The use of a saturated potassium chloride salt bridge should greatly minimize the error that might result from a secondary, diffusion potential. Further, the object was merely to obtain comparative potential measurements. For this purpose the procedure gave concordant results.

In the first of these experiments the milk, contained in the quart bottles was heated gradually by raising the temperature of

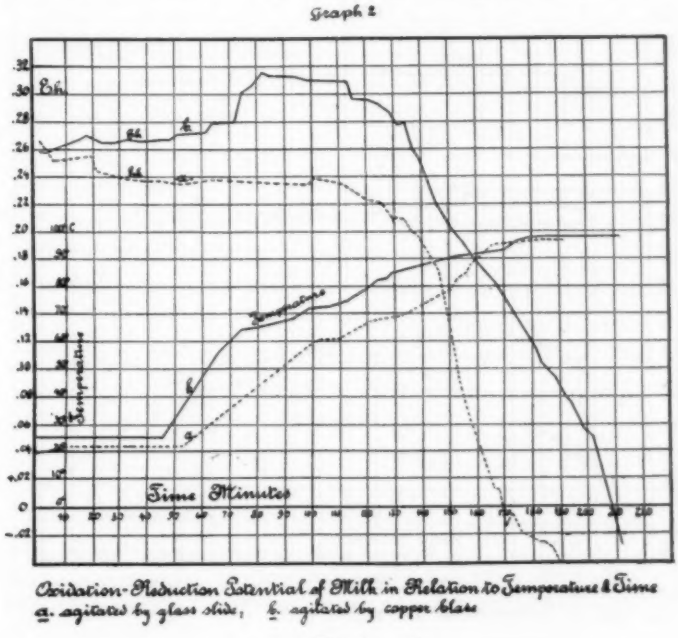
Graph 2
Eh.
Temperature
Time Minutes
Oxidation-Reduction Potential of Milk in Relation to Temperature & Time
a. agitated by glass slide, b. agitated by copper blast

FIG. 3

the water-bath. The changes in the potential in the milk, measured at frequent intervals together with the temperature of the milk, are illustrated in figure 3. Because the rate of temperature increase was not uniform, both temperature and potentials are plotted against time; direct plotting of potentials against temperature would be simpler but would not present the facts as

accurately. The relation between potentials and temperature can be seen by comparing the two on the same "time" ordinate. In one case (curve *a*) the milk was agitated with a glass slide, in the other (curve *b*) with a copper test piece attached to the usual agitating device. From this and similar experiments the following conclusions may be drawn:

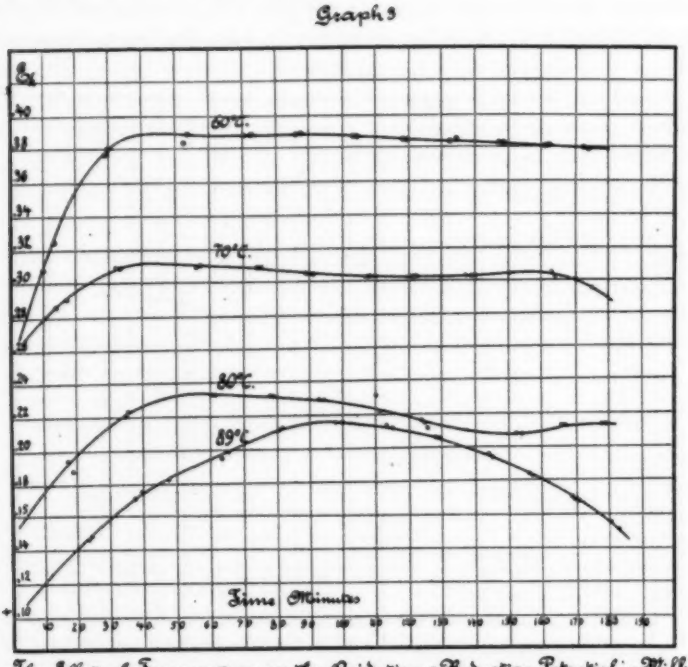

FIG. 4

1. Up to a temperature of about 65°C., the oxidation-reduction potential remains substantially constant with increases in the temperature (curve *a*).

2. When the milk is agitated by a copper blade, the potential was found to increase rapidly after seventy-two minutes when the temperature was about 63°C. Upon further increase of the

temperature up to 75°C., the potential remained almost constant.

3. At temperatures above 70°C., (or 75°C. in case of agitating with a copper blade) the oxidation-reduction potential of milk decreases rapidly, and finally reaches negative values somewhat after boiling temperature has been reached.

4. The presence of dissolved copper renders the potential more positive (compare curves *a* and *b*).

TABLE 6

The solubility of copper in milk in relation to the oxidation-reduction potential

SAMPLE	TEMPERATURE DURING EXPOSURE	TIME OF EXPOSURE	GAS TREATMENT	POTENTIAL, E_h		COPPER IN MILK, MEAN OF 2 DETERMINATIONS IN MGM./L.	COPPER DISSOLVED IN MGM./DM.²/HR.*
				Maximum	Final		
	°C.	minutes					
a	60	173	None	0.390	0.379	9.02	5.97
b	70	173 130†	None	0.311	0.295	8.17	5.39
c	80	173	None	0.224	0.203	5.50	3.60
d	90	173	None	0.206	0.140	5.24	3.42
				Initial	Final		
e	24.5	120	None	0.271	0.274	0.871	0.70
f	25.5	120	Carbon dioxide	0.159	0.039	0.368	0.21
g	25.5	120	Air	0.287	0.308	1.325	1.14
h	28.0	120	Oxygen	0.331	0.455	1.164	0.98

Experimental details:

Surface of copper: 25.8 cm.²

Amount of milk used: 500 cc.

Amount of milk ashed: 250 cc. for samples a–d; 500 cc. for samples e–h.

* Calculated under the assumption that the copper content of the original milk was 0.15 mgm. per liter.

† After one hundred thirty minutes the stirring cable broke; the milk of this sample was not agitated for the remainder of the experimental period.

Experiments of this type, in which the temperature of the milk was raised gradually, the potential was apparently dependent on both, the temperature, and the time. In order to determine the rôle of time, various temperatures were kept constant in the following experiments, and the potential changes with time were observed. (See figure 4.) The finding that the oxidation-

reduction potential decreases markedly at temperatures beyond 70°C., seemed a promising indication that this might be an important factor in explaining the marked decrease in copper solubility in milk beyond that temperature. Further experiments were therefore undertaken to study the oxidation-reduction potential of milk at high temperatures and to determine whether there is a relation between the potential and the amount of copper dissolved. The results of such experiments are presented in table

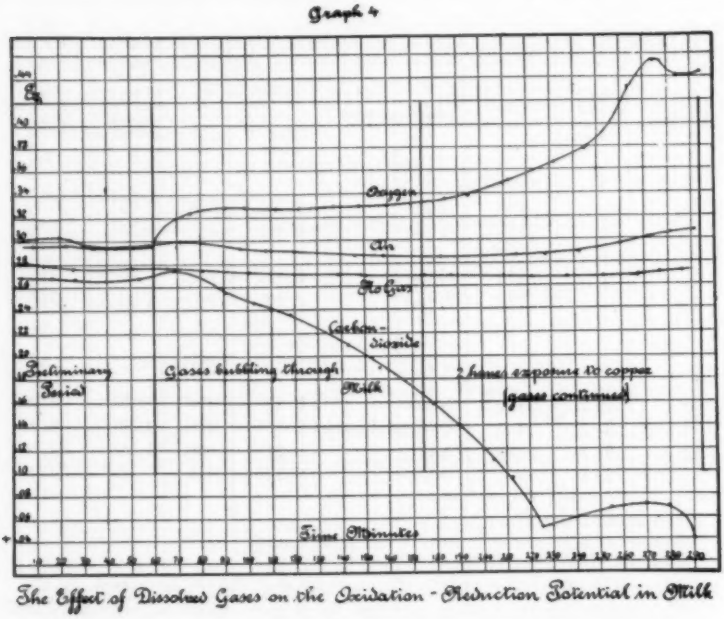

FIG. 5

6 and in figures 4 and 5. The details of the experiments are apparent from the description of the general procedure in measuring the potentials, and from the details indicated in the table and graphs. The following conclusions seem justified:

1. At 60 and 70°C. almost constant potentials are obtained.

2. The amount of copper dissolved is related to the oxidation-reduction potential. The potential attained and the amount of

copper dissolved when oxygen was bubbled through the milk presents an exception to this conclusion, but in this case it was very evident that the copper was coated with an oxide film which undoubtedly acted as a protective coating.

3. Dissolved copper makes the potential more positive, as indicated in figure 4, especially in the sample where oxygen was bubbled through.

DISCUSSION OF EXPERIMENTAL RESULTS

The experiments reported above have concerned themselves with (*a*) acidity, (*b*) dissolved gases, (*c*) temperature, (*d*) preheating time and temperature, and (*e*) oxidation-reduction potential, as factors in determining the amount of copper dissolved by milk. It is now of interest to correlate the isolated conclusions, which were drawn at the end of each experiment, in an attempt to gain a more comprehensive and fundamental understanding of the problem.

The data reported here show that an increased acidity causes a decrease in the solubility of copper in milk. This is contrary to the general belief and previous statements. Rice and Miscall (12) concluded: "Milk slightly sour dissolves but little more copper than normal milk." However, they performed only two experiments and in one of them the amount of copper dissolved in milk was actually less at the higher acidity. This finding that increased acidity causes a decreased copper solubility in milk is in harmony with the conclusions reported by Speller (13) as to the relative importance of the factors that are involved in the corrosion of steel in water within the range pH 4.3 to 10; he enumerates the factors in the order of their importance as follows:

1. Amount of dissolved oxygen present at the metal surface.
2. Protective coatings.
3. Hydrogen-ion concentration.
4. Composition of the metal.
5. Hydrogen overvoltage.

The importance of reaction is subordinate to the effect of dissolved oxygen and protective coatings. As will be indicated

later, dissolved oxygen exerts its effect through its influence on the oxidation-reduction potential. It is known that an increase in acidity causes the oxidation-reduction potential to become more negative, and it is probable that the decreased copper solubility with increased acidity is caused by this effect.

The experiments on the effect of oxygen, air and carbon dioxide on copper solubility confirmed, in a general way, the findings of previous workers. The quantitative relationship of the effect of these gases necessarily was different, because of the improved experimental method of exposing copper to milk. The effect of the dissolved gases on the oxidation-reduction potential indicates that they influence copper solubility through their influence on the oxidation-reduction potential. Oxygen affects the oxidation-reduction potential directly; the bubbling of oxygen, air, and carbon dioxide through the milk affects copper solubility only in so far as such a procedure determines the potential by the amount of oxygen remaining dissolved in the milk.

In the experiments on the effect of temperature it was found that copper solubility increased, attained a maximum value, and then decreased markedly with increases in temperature. This agrees, in general, with the findings of Quam (9) and Miscall et al. (7), but in the detailed results a number of differences are found. These differences, as well as the general temperature-solubility relationship, can now be explained.

The increase in copper solubility with temperature is expected and up to 50°C. follows the temperature-reaction-velocity equation. The oxidation-reduction potential remains substantially constant up to this temperature, and apparently is not a limiting factor. At temperatures somewhat above 50°C. the rate of copper solution is such that the oxidation-reduction potential becomes a limiting factor thereby causing a deviation from the temperature-reaction-velocity curve. In the experiments here reported, it was found that the oxidation-reduction potential of milk decreases markedly when milk is heated to temperatures above 70°C. As a result, the oxidation-reduction potential becomes a limiting factor to such an extent that copper solubility actually decreases with further increases of the temperature.

The temperature of maximum solubility reported by Quam was higher (85°C. to 90°C.) than that found above. From the present work it appears that such a high temperature of maximum solubility was to be expected in connection with their experimental procedure where the milk was exposed to the copper in the form of a thin film. Under such conditions the amount of oxygen dissolved in the milk in contact with the copper was obviously higher than what it would have been in the main body of the milk. The high temperature of maximum solubility was therefore not caused by the shorter time of exposure as used in their tests (thirty minutes as compared to one-hundred and twenty minutes), and the lower temperature of maximum solubility reported by others was not due to chemical changes which would supposedly occur in milk when heated to such temperatures for times longer than thirty minutes, as Quam et al. suggested. The same line of reasoning as above also explains the higher copper solubility which was observed by these authors at boiling temperature. On the other hand, the lower temperatures of maximum solubility reported by Miscall et al. were also to be expected because in their experiments no agitation was provided to keep the gaseous composition of the milk uniform and constant during the experiments.

The fact that the temperature of maximum solubility was always found to be 70°C., and that in previous work with other methods different temperatures of maximum solubility were found, indicates that this temperature is largely dependent upon the factors which determine the rate of oxygen diffusion to the metal surface (agitation, aeration, etc.). The procedure which was used in this work in arriving at the conclusion that the temperature of maximum solubility of copper in milk is at 70°C., controlled these essential factors; at the same time these conditions simulate conditions that obtain in cases of submerged corrosion under practical conditions.

The observations on copper solubility reported by Miscall et al. were difficult to explain, but in addition they suggested that the factors which condition copper solution in milk are exceedingly complex. The fact that they found a temperature of maximum

solubility not only in raw milk, but also in evacuated milk, or in milk saturated with oxygen, or with carbon dioxide, and especially the fact that in pasteurized milk a maximum solubility is obtained at about the same temperature as is obtained with raw milk, led these authors to conclude that oxygen can not account wholly for the copper solubility in milk, and that there is a reaction over which oxygen has no control. The inconsistencies and lack of correlation in their results are in all probability due to (*a*) lack of agitation in their procedure, (*b*) uncertainty in the amount of oxygen remaining in the milk as the result of the various treatments of the milk, referred to above. The length of time that is required to change the oxidation-reduction potential by passing carbon dioxide through the milk, suggests that more time is required for the removal of oxygen from milk, than is generally assumed.

It has been suggested that an oxygen transmitting enzyme is active in copper solution in milk, and that the break in the temperature-solubility curve at 70°C. and the reduced solubility in preheated milk is due to the destruction of this enzyme. In view of the experimental work presented here, there is no justification for such an assumption, and the above phenomena can be explained on the basis of physico-chemical factors. The observation reported in table 5 (samples *m* to *p*), that the bubbling of carbon dioxide through milk which had been preheated to various temperatures, did not entirely eliminate differences in copper solubility, may be explained by the slow action of carbon dioxide in changing the oxidation-reduction potential, as shown in figure 4. This indicates that the main effect of preheating is the gradual expulsion of dissolved oxygen, thus lowering the oxidation-reduction potential.

In addition to the above, chemical changes may constitute a second factor, involved in the decreased copper solubility in preheated milk. The drop in the oxidation-reduction potential with prolonged heating at 80 to 89°C. (fig. 4) suggests that organic milk constituents undergo chemical changes. It is known that lactose undergoes oxidation in milk at high temperatures (Whittier and Benton (15); this constitutes a mode of oxygen removal

that supplements the expulsion of this gas on account of decreased solubility at higher temperatures. Besides the removal of oxygen by chemical union and expulsion from solution, a drop in the potential may be caused by chemical changes that produce substances with reducing properties; for example, it is known that hydrogen sulphide is evolved when milk is heated at high temperatures. Bubbling oxygen through preheated milk will restore the depleted oxygen content, but the effects of certain chemical changes, probably of the type indicated above, are apparently not so readily eliminated. This would account for the decreased copper solubility in previously boiled milk, even when oxygen is bubbled through it (table 4).

In view of the important rôle of oxygen in the solution of copper in milk, analyses to determine the amount of dissolved oxygen under various conditions may appear important. However, no such analyses were made in this work. While they would be of interest, they would not necessarily furnish precise proof of the relationship of dissolved oxygen to copper solubility. The effect of oxygen is operative through its influence on the oxidation intensity which is conditioned by other factors besides the absolute amount of oxygen dissolved. Therefore, the direct measurement of the oxidizing intensity by means of the oxidation-reduction potential was used instead of gas analyses.

The various aspects of the problem as revealed in the present study lead to the conclusion that the solution of copper in milk actually represents a case of metal corrosion in dilute acid solution. The relative importance of the factors that apply in such cases, as enumerated by Speller (13), appear to apply to the present problem. This becomes even more convincing as we compare the copper solubility in milk with the corrosion of metals in other media. Even in strong acid solutions the rate of metal solution is dependent upon the oxygen content of the liquid, as shown by Whitmann and Russell (14). These investigators determined the rates of copper solution in media in equilibrium with (*a*) pure oxygen, and (*b*) air. The solubility with oxygen exceeded that obtained with air, in various media at 22°C. by the following multiples:

6 per cent sulphuric acid	3.7
15 per cent sulphuric acid	3.8
20 per cent sulphuric acid	3.8
6 per cent acetic acid	5.1
6 per cent hydrochloric acid	10.7

They attributed the high solubility ratio in hydrochloric acid to the increased oxidizing capacity of the solution caused by the accumulation of cupric ions.

In this connection it is of interest to note that the ratio of copper solubility in milk when in equilibrium with oxygen to that in equilibrium with air was found as follows:

2.1 at room temperature (table 4, sample *a*, and figure 2 at 20°C.).
2.9 at pasteurizing temperature (table 2, sample *k*, and figure 1, curve *d*, at pasteurizing temperature).
3.1 at pasteurizing temperature (table 4, samples *b* and *f*).

This lower ratio is indicative of the poising effect, or of the slower rate of oxygen diffusion in the milk, which is apparently more pronounced at lower temperatures.

Beyond the scope of this study, the observations on the change of the oxidation-reduction potential in milk are of general interest. Oxidative processes in dairy products are known to influence their flavor and keeping quality, and studies to bring out the relationship of oxidation-reduction potentials to oxidative changes, appear promising.

SUMMARY AND CONCLUSIONS

1. In the review of previous work, the lack of concordant results was attributed to faulty procedure,—lack of agitation, and inadequate control of the temperature and dissolved gases. A procedure was developed to overcome these objections.

2. The effect of acidity, dissolved gases, and temperature on the solubility of copper in milk were studied. The effect of velocity was not studied, but it was controlled and kept uniform in all the experiments.

3. Higher acidities markedly decreased (rather than increased) copper solubility in milk, while slight increases in acidity had no effect.

4. The effect of dissolved gases was studied by bubbling them through the milk during the entire exposure. It was found that carbon dioxide markedly decreased, air slightly increased, and oxygen markedly increased the copper solubility in milk. Prolonged treatment of the milk with oxygen previous to exposure led to the formation of a protective oxide coating.

5. Temperature greatly affected copper solution in milk. The maximum solubility was always found at 70°C. whether the time of exposure was thirty minutes, one hour or two hours. In high acid milk the maximum was also at 70°C., but was appreciably lower than in sweet milk. At boiling temperature copper solubility was about the same as at room temperature.

6. Up to 50°C., the temperature-copper-solubility curve followed the temperature-reaction-velocity curve. At higher temperatures, the curves diverged, less than the expected amount of copper being dissolved.

7. Preheating of milk to various temperatures above 70°C. decreased copper solubility in such milk. The effect of preheating was greater as the temperature and time of preheating increased.

8. The oxidation-reduction potential of milk was found to be related to the solution rate of copper and the various factors that affect it.

9. Up to a temperature of about 65°C., the oxidation-reduction potential remained substantially constant; above 70°C. it decreased rapidly. With prolonged holding at 60 and 70°C., the potential remained constant. The presence of dissolved copper made the potential more positive. Increased acidity is known to make the potential more negative.

10. In the discussion it is shown how the observations on the oxidation-reduction potential may explain the mechanism through which acidity, dissolved gases, temperature and preheating affects copper solubility.

11. Copper solution in milk is a typical case of metal corrosion. The same factors in the same relative order of importance apply to copper corrosion by milk as apply in general to metal corrosion by weakly acid or alkaline solutions.

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THE RELATIVE ANTINEURITIC (VITAMIN "B") POTENCY OF CERTAIN MILK DERIVATIVES*

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Although the multiple character of the "vitamin 'B' complex" is now clearly recognized, the lack of universally accepted methods for general assay purposes has retarded the accumulation of data showing the relative distribution of the different factors in various foods. The published investigations however, clearly indicate a relative abundance of vitamin "B"—the antineuritic factor in cereal grains, and somewhat less in animal products including milk, whereas, the reverse is true in regard to vitamin "G"—the anti-pellagric or growth-promoting factor. Various investigations (1–4) have indicated a relatively low vitamin "B" content of milk, and it may be concluded from the data available that this is the limiting factor causing retarded growth in rats when limited amounts of milk are fed as the sole source of this vitamin. Aside from the effect of vitamin "B" on growth, its specificity for curing or preventing polyneuritis in pigeons, rats and man is generally recognized and much of the available data concerning the distribution of this vitamin have been obtained by suitable technique for the cure and prevention of this disease.

EXPERIMENTAL

A comprehensive study of the nutritive properties of a water-soluble milk vitamin concentrate, concerning which a brief preliminary report has been published (5), has necessitated, as a pre-requisite to further studies, a determination of its antineuritic potency. The results of these determinations as well as those obtained from refined lactose, dry whey and rice polishings are recorded herein. The curative technique with the use of pigeons

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as recommended by Kinnersley, Peters and Reader (6) (7) was used.

The test substances were rice polishings of commercial grade, milk sugar refined to a degree well beyond the requirements of the U. S. Pharmacopeia standards, whey resulting from a rennet precipitation of casein, subsequently dried by a spray process commonly used in Europe, and the vitamin concentrate having a gross composition computed to the dry basis as follows: Lactose, 52.01 per cent; ash, 30.84 per cent; and nitrogen, 1.54 per cent.

The pigeons used for test purposes were kept on a complete standardized ration for a period of thirty days before placing on the restricted diet prescribed for the development of polyneuritis. This complete diet consisted of 16.6 parts whole buckwheat; 16.6 parts whole cracked corn; 16.6 parts millet; and 50 parts whole wheat. At the end of the thirty-day period on this ration the birds were confined in wire cages with screened bottoms in a warm room in the laboratory building. A restricted ration consisting only of autoclaved polished rice was given ad libitum with 1 cc. of cod liver oil fed weekly by pipette. Those birds which did not develop characteristic opisthotonos or head retraction and convulsions within thirty-five days on the polished rice diet were not used for assay purposes. Many of the birds subjected to the restricted diet developed symptoms between the thirty-fifth and forty-second day, but since these were usually in a weakened condition the reactions from the test substances were irregular and inconsistent. About 5 per cent of the pigeons developed non-typical symptoms, emprosthotonos before the thirty-third day. The results from these non-typical cases were neither consistent nor satisfactory and after preliminary trials with such specimens these cases were discarded. Only 40 per cent of the total number of pigeons given the rice diet were available for test purposes as determined by the development of typical polyneuritis symptoms within the prescribed time. With but few exceptions the afflicted birds were given the second dose of test substance if recovery resulted from the first dose. After the second dosage and at the third onset of convulsions they were given a liberal dose of rice polishings and a yeast tablet

consisting of about 250 mgm. and then returned to the complete stock ration for recuperation and recovery.

The test substances were given in limited amounts of water by pipette in all cases within one-half to three hours after the appearance of unquestionable symptoms. When the substance was sufficiently potent recovery was rapid, and with the exception of border-line doses, subsidence of the convulsions and head retraction was brought about within five hours. In many such cases the birds were able to stand erect and consume food and water. In those instances wherein only border-line doses were administered recovery was never complete. In these cases severity of the convulsions usually subsided, head retractions became less pronounced, but the birds continued to lose weight. Very few of these cases recovered to a degree which permitted voluntary food consumption. Forced feeding was resorted to in a few instances, but in all such cases death ultimately ensued with or without re-occurrence of convulsions and opisthotonos.

The records from typical cases wherein reaction time and consistency of results were most regular are shown in table 1. Plate 1 shows the typical reaction of pigeon 46A afflicted with polyneuritis on the nineteenth day. Plate 2 shows the same pigeon five hours after receiving the first 2-gram dose of the water-soluble milk vitamin concentrate. Protection was afforded for one hundred twenty hours by this dosage. The records show that 0.5 gram of rice polishings and 2 grams of the water-soluble milk vitamin concentrate contained substantially the same amount of the antineuritic vitamin. Inconsistencies in the period of protection conferred by the milk vitamin concentrate and dry whey when computed to the day dose requirement are apparent, and it may be considered that while the different levels of these substances exhibited definite antineuritic properties, the more valid basis for computing relative potency must be on those quantities which consistently gave the longest period of protection. A minimum protective period of three days is considered necessary by some investigators (6) (7) (8). Such a basis of appraisal would indicate that 1 gram of rice polishings contained about four times the antineuritic potency of 1 gram of the dry matter of

TABLE 1

Antineuritic potency of test substances as determined by the curative technique with pigeons

PIGEON NUMBER	REACTION TIME	TEST SUBSTANCE	AMOUNT OF TEST SUBSTANCE	PROTECTION PERIOD		REMARKS
				After first reaction	After second reaction	
	days		*grams*	*hours*	*hours*	
23	19	Lactose	0.5	None		No indication of curative effect
46	19	Lactose	0.5	None		No indication of curative effect
83	27	Rice polishings	0.5	110		Improvement within 5 hours—cured
89	31	Rice polishings	0.5	51	48	Improvement within 5 hours—cured
59	32	Rice polishings	0.5	144		Improvement within 5 hours—cured; died after second reaction
61	35	Rice polishings	0.5	144		Improvement after 5 hours—cured; died after second reaction
7615	28	Rice polishings	0.5	140	144	Improvement within 5 hours—cured
741	25	Milk vitamin concentrate	0.75			Only slight improvement—cure not effected
700	33	Milk vitamin concentrate	0.75			Only slight improvement—cure not effected; died after 56 hours
716	33	Milk vitamin concentrate	0.75			Only slight improvement—cure not effected; died after 74 hours
712	28	Milk vitamin concentrate	0.75			Mild reaction—only slight improvement; died after 7 days
69	24	Milk vitamin concentrate	1.50	56	48	Improvement within 5 hours—cured
1056	24	Milk vitamin concentrate	1.50	50	55	Improvement within 5 hours—cured
781	25	Milk vitamin concentrate	1.50	70	96	Improvement within 5 hours—cured

23A	19	Milk vitamin concentrate	2.00	120		Improvement within 5 hours—cured
46A	19	Milk vitamin concentrate	2.00	120	120	Improvement within 5 hours—cured
8	18	Milk vitamin concentrate	2.00	120		Improvement within 5 hours—cured
23B	18	Milk vitamin concentrate	2.00	112		Improvement within 5 hours—cured
55	15	Dry whey	1.50	38	76	Improvement within 5 hours—remained very weak
1056A	22	Dry whey	1.50	73	27	Improvement within 5 hours—remained very weak
46B	24	Dry whey	1.50	26	20	Improvement within 5 hours—remained very weak
69A	21	Dry whey	1.50	62	38	Improvement within 5 hours—cured
713	26	Dry whey	1.50	97	42	Improvement within 5 hours—cured
1345	26	Dry whey	1.50	96		Improvement within 5 hours—not completely cured
1342	32	Dry whey	3.00	88	24	Improvement within 5 hours—cured
1346	33	Dry whey	3.00	48	45	Improvement within 5 hours—cured
1355	34	Dry whey	3.00	72		Improvement within 5 hours—cured
1056B	18	Dry whey	3.00	102	47	Improvement within 5 hours—cured
69B	22	Dry whey	3.00	65	52	Improvement within 5 hours—cured
55A	15	Dry whey	3.00	76		Improvement within 5 hours—cured

the water-soluble milk vitamin concentrate, and eight or slightly more times that of the same amount of dry whey.

A preliminary report (5) on the growth-promoting properties of the water-soluble milk vitamin concentrate indicated that slightly more than 1 gram per day of this substance was required to supply the vitamin "B" complex requirement for the normal growth of white rats. It will be shown in a subsequent report that the limiting factor in these earlier studies was vitamin "B" and not vitamin "G." Other investigators (3) (9) have shown that 3 to 3.5 grams of milk solids per day is required to supply the vitamin "B" requirement of the growing rat. A previous communication (10) which came to us recently stated that a sample of the water-soluble milk vitamin concentrate, now under investigation at another laboratory, contains about four-tenths as much vitamin "B" as a good grade of yeast. This assay was made by the pigeon maintenance technique with a sample of the concentrate from which about 40 per cent of the inert material contained in the product reported in this paper had been removed.

SUMMARY

The vitamin "B" assay of a water-soluble milk vitamin concentrate from which the fat, casein, albumen and a substantial proportion of the milk sugar had been removed showed an antineuritic potency substantially 25 per cent of that of rice polishings, according to the curative technique with pigeons.

Desiccated whey solids, assayed according to the curative technique with pigeons showed an antineuritic potency of not more than 10 to 12 per cent of that of rice polishings, or slightly less than one-half that of the water-soluble milk vitamin concentrate.

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PLATE 1

PIGEON 46A WITH POLYNEURITIS REACTION AFTER NINETEEN DAYS ON POLISHED RICE DIET

PLATE 2

PIGEON 46A FIVE HOURS AFTER RECEIVING A 2-GRAM DOSE OF THE WATER-SOLUBLE VITAMIN CONCENTRATE OF MILK

Characteristic polyneuritis symptoms have disappeared; protection lasted for five days.

PLATE 1

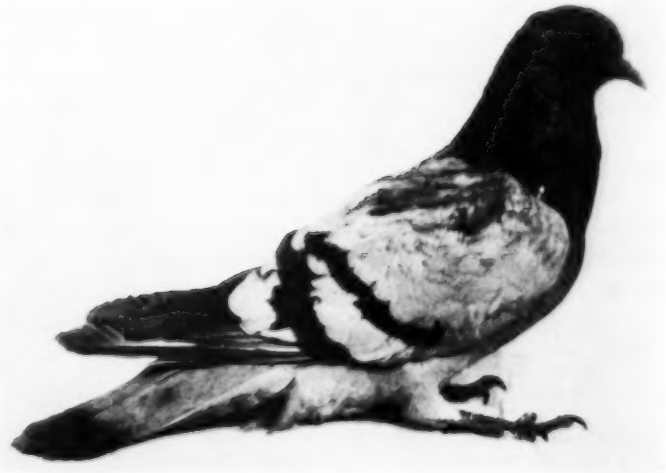

PLATE 2

MALT FLAVOR IN RAW MILK PRODUCED BY MICROCOCCUS*

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During the past summer (1930) a malt-like flavor was detected in some of the raw milk delivered at the University Creamery. So objectionable was this flavor that some of the affected milk could not be used for market milk purposes. Pasteurization did not remove the defect entirely.

As the acidity of this milk usually was not over 0.18 per cent the flavor was first attributed to feed the cows had eaten. A survey of the pasture and feed lot on one of the farms sending in the malt flavored milk, however, failed to reveal any weeds or other feeds that might possibly cause the defect. The milk was stored over night in cans in an outside galvanized iron tank away from feed odors. It was found that the first milk through the milking machine developed much more of the off-flavor. It was also noted that the night's milk only was affected at the time of delivery to the plant at about 9 a.m. The milking machine, according to the farmer's statements, was washed once a day and was rinsed with a sodium hypochlorite solution before using. Examination of the machine showed the rubber parts to be in rather poor condition, being full of small crevices on the inside. Upon replacing these tubes with new ones, the flavor disappeared for a few days but later returned suggesting the possibility of bacteria as being the cause of the malt flavor.

EXPERIMENTAL

A malt flavor in milk and butter was studied by Hammer and Baker (1) and found to be caused by *Streptococcus lactis var. maltigenes*. They report the flavor and odor as being very apparent in certain raw milks and creams entering dairy plants at acidities under 0.2 per cent. They also state that the defect was found to be more evident in summer than winter. The

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optimum temperature for growth of the bacteria and development of the flavor was given as 37°C.

In our studies an organism (culture 20) was isolated from some of the malt-flavored milk, which, when grown in pure culture at 68°F., reproduced the flavor defect to some extent. This organism is not the same as the one reported by Hammer and Baker, being a micrococcus of aureus type.[1] It was noted that in all the milk that had a pronounced malt flavor a spore-forming organism of the *B. subtilis* group was commonly present (culture 1). Although culture 20 alone will produce a slight malt flavor, the presence of culture 1 seems necessary for a distinct flavor development, indicating an associative action between the two orgaisms. It may be that enzymes produced by culture 1 make it possible for certain proteolytic actions of culture 20 to progress more rapidly. This was evidenced by the fact that the addition of pepsin and rennet to separate inoculations of culture 20 resulted in a decrease in time required for the malt flavor to appear.

It was observed that the most pronounced malt flavor occurred in those raw milks in which there was a predominance of cultures 1 and 20. The flavor was not so evident when acid-forming bacteria were present. It was also noticed that in some instances a strong malt flavor would be present, but would later disappear after the milk stood at room temperature for a few hours.

OCCURRENCE

Plant men in various parts of the country during the past summer and fall have complained of a feed-flavor in raw milk which, in some cases, they attributed to the cows eating weeds, because of the shortage of grass pasture. Examination of some such milk by one of the authors revealed the fact that the flavor was identical to the malt-flavor of bacterial origin. A study made at a central Illinois dairy showed that the milk delivered by twenty of the three hundred producers had the malt-flavor. Organisms similar to culture 20 in appearance and in ability to

[1] A culture of *S. lactic var. maltigenes* secured by the authors from Hammer when grown in pure culture produced a very potent malt flavor identical to the one produced by the micrococcus.

produce malt flavors have been isolated from the milk delivered by twenty of the twenty-two University milk patrons. However, the milk from only three of these patrons has been a serious source of the flavor.

Malt-flavor producing micrococcus organisms were found in aseptically drawn milk from the udders of twenty-four apparently healthy cows on three farms producing milk containing the off-flavor. The companion organism (culture 1) was found to be

TABLE 1

Description of cultural characteristics of organisms producing malt-like flavor in milk

	CULTURE 20	CULTURE 1 (SUBTILIS GROUP)
Classification	Micrococci	Bacillus
Form—Arrangement	Packets—coccus	Long rods in chains
Sporulation	−	+
Motility	−	+
Color	Orange	Dirty white
Occurrence	Udder	Hay, soil
Colony formation	Small punctiform 1 mm.	Flat spreading 6 mm.
H_2S from peptone	−	−
Nitrites from peptone	−	−
Nitrites from nitrates	+	+
Starch hydrolysis	−	+
Litmus milk	Very slow acid curd	Slightly acid rennet curd
Dextrose	Acid, no gas	Acid, no gas
Lactose	Acid, no gas	Acid, no gas
Sucrose	Acid, no gas	Acid, no gas
Glycerol	Acid, no gas	Neutral, no gas

rather commonly distributed on utensils and in the soil of the barnyards.

CULTURAL CHARACTERISTICS

The cultural characteristics of the two organisms (1 and 20) responsible for the malt-flavor in milk are given in table 1.

When a purified casein solution was inoculated with cultures 1 and 20, malt-like odors were obtained suggesting a protelytic action. In methylene blue milk, reduction took place in one

instance in two hours and fifteen minutes with a count of 700,000 and in another case in one hour and forty-five minutes with a count of 1,600,000.

TABLE 2

Effect of pasteurization upon culture 20

Sample	*Bacteria per cc.*
Raw	8,400,000
Heated to 122°F.	6,200,000
" " 131°F.	5,600,000
" " 140°F.	4,800,000
" " 142°F.	3,000,000
Held at 142°F. for 2 minutes	1,056,000
" " 142°F. " 5 "	420,000
" " 142°F. " 10 "	150,000
" " 142°F. " 15 "	1,000
" " 142°F. " 20 "	No colonies

TABLE 3

Relation of temperature to the development of malt-like flavor in milk

TEMPERATURE	COUNT AFTER FOUR HOURS' INCUBATION	TIME WHEN FLAVOR FIRST DETECTED	COUNT WHEN PRONOUNCED FLAVOR FIRST DETECTED
°F.	*per cc.*		*per cc.*
60	(1) 50,000 (20) 630,000	No malt-like flavor even after 72 hours (cheesy odor)	3 days total count of 15,000,000
68	(1) 180,000 (20) 680,000	20 hours	(1) 920,000 (20) 4,800,000
85	(1) 111,000 (20) 920,000	9 hours	(1) 880,000 (20) 9,500,000
100	(1) 150,000 (20) 1,420,000	6 hours	(1) 800,000 (20) 9,000,000

THERMAL DEATH POINT (ABSOLUTE)

That a micrococcus causing a malt flavor in milk may be responsible for diarrhea and other intestinal disturbances was suggested by Ramsey and Tracy (2). In view of the fact that our data suggest that this type of organism may be of rather common occurrence, it seems that its resistance to pasteurizing tempera-

tures would be of importance from the public health standpoint. As shown by data in table 2, the organism is destroyed by ordinary pasteurizing procedures. Culture 1, however, was found to survive pasteurization and was not completely destroyed even at boiling temperatures.

FIG. 1. PHOTOMICROGRAPH OF CULTURE 20 (MICROCOCCUS) AND CULTURE 1 (BACILLUS) WHICH WERE FOUND TO PRODUCE A MALT FLAVOR IN MILK

Organism 20 will produce the flavor when grown in pure culture but when culture 1 is present a much more pronounced malt flavor results. (Magnification 1130.)

IMPORTANCE OF TEMPERATURE IN DEVELOPMENT OF MALT FLAVOR

In studying the occurrence of the flavor in raw milk delivered at the plant, it was noted that the defect was most common when the nights were warm. Table 3 shows the relation existing between temperature and the development of the flavor in milk.

Although the defect occurred sooner in samples held at the higher temperatures (85° to 100°F.), the most characteristic flavor was in the milk held at 68°F. It is interesting to note that the flavor failed to develop in the milk held at 60°F. even after seventy-two hours with a count of 15,000,000, which probably explains why the flavor is most common in summer months.

SUMMARY AND CONCLUSIONS

Data have been presented to show that an objectionable malt-flavor in milk may be caused by the action of a micrococcus of aureus type (culture 20). It was also found that when an organism of the *B. subtilis* group (culture 1) was also present, a much more pronounced malt-flavor resulted. The presence of acid-forming bacteria retarded the development of the flavor.

The flavor defect was found to be rather common in raw milks entering certain milk plants during the summer months. Not all the samples containing the suspected organisms had the flavor at the time they arrived at the plant. In some instances, the flavor disappeared from samples held at room temperature.

Utensils were found to be a direct source of the large rod (culture 1), while the coccus forms (culture 20) were found in the udders of cows in suspected herds.

Neither the coccus or the rod-shaped organisms were found to produce gas. They were proteolytic in their action. Pasteurization of pure cultures of the coccus organisms (culture 20) at 142°F. resulted in their death after twenty minutes, whereas, the rod-shaped organisms (culture 1) were found to survive even higher temperatures.

The malt flavor was found to develop most rapidly in milk held at 85°F. and 100°F. although a more characteristic flavor was noted in the milk held at 68°F. The defect failed to develop in milk held at 60°F. for three days.

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A STUDY ON METHODS FOR DETERMINING NUMBERS OF MOULDS AND YEASTS IN BUTTER*

I. THE RELATION OF THE pH OF THE MEDIUM

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The rapid development in the use of the routine mould and yeast analysis of butter as an index of the sanitary conditions and the efficiency of manufacturing processes in the creamery make it desirable that uniform procedures be adopted if the results from different laboratories are to be comparable. In Canada, uniform methods for mould and yeast analysis are especially necessary due to provincial mould and yeast competitions and to the adoption of the modified score card (1) for exhibition butter.

The importance of adopting uniform procedures for the microbiological analysis of butter was recognized by the American Dairy Science Association and the report (2) of the sub-committee studying this question has been published recently.

In a study of the media commonly used by various workers in analyzing butter for moulds and yeasts, Hood and White (3) found that one of the most variable factors was the final pH of the media which ranged from 3.4 to 4.6. When the pH value of the medium was higher than 3.8 numerous bacterial colonies were present on the plates which increased the total number of colonies greatly, made counting difficult, and introduced inaccuracies in the yeast count. From the results of this study the authors recommended a final pH of 3.5 $\pm$ in the medium for Canadian laboratories.

Clerkin (4) also found that as the pH value of the medium varied from 3.5 to 6.0 the total number of colonies of bacteria and yeasts increased but that the ratio of bacteria to yeasts increased with the higher pH values. This author concludes that

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the medium used must have a pH of 3.5 ± before the plate counts obtained could, with safety, be regarded as consisting entirely of yeasts.

Hammer (5) states that 1 cc. of 1 per cent tartaric acid solution per plate (8 to 10 cc. medium) gives satisfactory results but does not limit the growth of bacterial colonies, and that with greater amounts of acid there is a gradual decrease in the number of yeasts developing.

The divergence of opinion among the various workers, as evidenced by the final pH of the medium they employed, suggested that a study be made of the relation of the pH of the medium, firstly, to the growth of pure cultures of yeasts isolated from butter, and secondly, to yeast counts as obtained from butter itself.

METHODS

For the first part of the study, 63 cultures of yeasts were isolated from numerous samples of commercial and exhibition butter representative of all parts of Canada. The yeasts were isolated from plates poured with a malt agar medium pH 5.5 ± and picked to malt agar slopes. For purification purposes the cultures were then transferred to beer wort broth and high dilutions of the broth culture were plated using malt agar. Well isolated yeast colonies were transferred again to malt agar slopes at pH 5.5 from which further transfers were made during the investigation.

Before plating, the yeasts were grown in malt extract broth pH 4.8 ± for twenty-four hours at 25°C. at which time all cultures had produced good growth. Suitable dilutions were then made by transferring one loopful of the twenty-four-hour malt broth culture to a sterile 99 cc. water blank, and 1 cc. of this dilution was used for plating.

For the second part of the investigation 50 samples of butter were analyzed and included both pasteurized and unpasteurized butter representative of different grades according to Canadian standards. A preliminary mould and yeast count of the butter was made and then a dilution of either 1:10, 1:100, or 1:1000 was used for plating according to the preliminary count.

Both the pure yeast cultures and butter samples were plated on dehydrated malt agar (Difco) as this medium had been previously recommended by Hood and White (3) in their tentative methods for mould and yeast analysis. The medium was acidified to pH values of 3.0, 3.4, 3.8, 4.2 and 4.6 just before plating with a 5 per cent solution of lactic acid (U. S. P. 85 per cent). A LaMotte colorimetric hydrogen ion testing set was used to standardize the pH of the medium using bromphenol blue as an indicator. These pH values were selected because they represented the range of the final pH of the different media used by various workers.

For both yeast cultures and butter samples, plates were poured in duplicate for each pH value of the medium and incubated at 25°C. for five days. Counting was done with the naked eye and the results expressed as the average counts of the duplicate plates.

Before counting, the plates from the butter samples were carefully examined with a good hand lens to pick out if possible any bacterial colonies. Only colonies that appeared to be yeasts were counted and recorded. After counting, a microscopic examination of stained smears of 12 colonies from each plate was made. These colonies were picked from a representative area of one plate of each sample, and were typical of all colonies that were counted in this area. When bacterial colonies were found, the ratio of bacterial colonies to yeast colonies was considered to be representative of the whole plate and the counts were corrected accordingly.

RESULTS FROM PLATING PURE CULTURES OF YEASTS

In table 1 the average counts of duplicate plates for each pH value of the 63 yeast cultures used in this investigation are tabulated.

The results in table 1 show that the number of colonies from individual yeast cultures growing at the different pH values of the medium compare favourably with one another when the limitations of the plate method are taken into consideration. For cultures 15, 24, 27, 55 and 62 there was a noticeable decrease

TABLE 1

Counts of yeast cultures on media of different pH values

NUMBER OF CULTURE	pH 3.0	pH 3.4	pH 3.8	pH 4.2	pH 4.6
1	159	157	169	186	167
2	230	206	234	206	231
3	18	18	20	17	19
4	386	362	346	364	350
5	246	210	227	216	219
6	225	232	222	251	237
7	454	343	424	385	389
8	201	212	189	203	205
10	296	352	338	310	318
11	452	487	493	404	434
13	168	181	171	179	176
15	8	16	17	23	19
17	68	77	71	80	80
18	63	73	64	62	71
19	93	107	119	111	108
20	236	232	206	223	224
21	244	248	296	270	272
22	136	136	147	119	127
23	110	123	130	127	136
24	64	129	166	186	180
25	55	47	60	68	68
26	41	38	42	39	48
27	1	26	23*	28	27
28	256	281	277	295	275
29	81	67*	75	86	84
30	644	662	641	654	670*
31	155	165	153	147	142
32	39	41	39	40	33
33	134	172	129	142	135
34	168	174	202	187	173
35	400	337	340	363	360
36	128	128	138	145	137
37	180	199	193	182	203
38	226	199	211	193	193
39	320	303	343	300	311
40	339	337	364	319	315
41	332	333	343	328	336
42	248	240	228	235	249
43	342	384	384	407	334
44	268	227	253	234	214
46	130	132	147	165	146
47	385	381	397	443	418
48	403	447	425	465	439

TABLE 1—*Concluded*

NUMBER OF CULTURE	pH 3.0	pH 3.4	pH 3.8	pH 4.2	pH 4.6
49	388	413	401	401	374
50	345*	361	389	353	333
51	346	354	357	348	335
52	222	212	228	252	231
53	315	342	330	312	317
54	200	190	182	174	164
55	177	584	588	573	582
56	360	416	387	420	409
57	254	231	251	257	236
58	704	707	736	702	722
59	629	692	651	656	651
60	106	118	98	125	105
61	437	441	476	444	453
62	0	327	323	335	303
63	137	139	119	125	165
64	662	626	663	664	640
65	199	202	209	228	212
66	330	325	326	327	318
67	713	689	707	647	673
69	200	196	212	213	213
Average of all cultures.....	252	266	271	269	265

* Counts for one plate only.

in the number of colonies on the medium with a pH of 3.0 but there was no significant difference in the counts of these cultures at pH 3.4 to 4.6, and for the remaining cultures there was no great variation in the counts at the different pH values.

The average counts at each pH of the medium for all cultures did not show any significant differences; the highest average count of 271 was obtained at a pH of 3.8 and the lowest average count of 252 was for a pH of 3.0, a difference of only 19 colonies. The difference between the average counts at pH values of 3.4 to 4.6 was only 6 colonies.

The variation between the high and low average counts obtained at the different pH values was only 7 per cent, while for individual cultures the greatest variation in the counts at pH 3.4 to 4.6 was 30.7 per cent for culture 24, and the smallest variation

was 2.8 per cent for sample 66. For 25 cultures there was a difference of less than 10 per cent between the high and low counts, and for 55, or 87 per cent, of the cultures the difference did not exceed 20 per cent.

The results also show that the high and low counts of individual cultures were obtained at all pH values of the medium. Ten of the cultures had high counts even when the medium had a pH of 3.0, while the low counts for 20 of the cultures were obtained at this pH value. However, as none of the media commonly used in analyzing butter for moulds and yeasts is acidified to pH 3.0, the counts obtained at this pH are not considered further but are of interest in that they indicate that many of the yeasts commonly found in butter are able to grow and develop

TABLE 2

Showing the distribution of the high and low counts of individual cultures according to the pH value of the medium

pH OF MEDIUM	NUMBER OF CULTURES WITH HIGHEST COUNTS	NUMBER OF CULTURES WITH LOWEST COUNTS
3.4	12	21
3.8	20	14
4.2	22	12
4.6	6	13
Total cultures.....	60	60

at a pH value lower than is commonly used for mould and yeast control work.

In table 2 a summary is given showing the distribution of the high and low counts for pH values of 3.4 to 4.6.

In addition to the 60 cultures summarized above, the high counts for three cultures were the same at pH values of 4.2 and 4.6, and the low counts for three cultures were the same at two different pH values.

These figures clearly show the even distribution of the high and low counts from the individual yeast cultures over the different pH values, and indicate that the yeasts used in this study were able to grow equally well over the range of pH studied.

After counting, all plates were examined carefully to determine

if the pH of the medium had any effect on the size of the colonies. In the case of ten cultures, representative surface colonies were measured and there was found to be no appreciable difference in the size at any pH value. To the eye, the subsurface colonies of some yeast cultures seemed to grow slightly larger at pH values of 3.8 to 4.6 than at pH values of 3.0 and 3.4, but with the majority of the cultures there was no apparent difference in the size of subsurface colonies.

There was a good development of the yeasts on all plates after five days at 25°C. and all colonies were discerned easily with the naked eye.

RESULTS FROM PLATING BUTTER SAMPLES

In table 3 the numbers of yeast colonies from butter samples are recorded which appeared on the medium at different pH values.

A study of the data presented in table 3 shows that for the majority of the samples there were no marked differences in the number of yeast colonies that developed at pH values of 3.4 to 4.6, and in many cases the counts at pH 3.0 were comparable to the other counts. These results also show that the maximum and minimum counts for individual butter samples occurred at all pH values of the medium, excluding the counts at pH 3.0 which is considerably below the range of pH of the media commonly used for routine yeast and mould analysis of butter.

One sample had the highest count at a pH of 3.4, 8 samples at a pH of 3.8, 19 at a pH of 4.2, and 22 at a pH of 4.6. The minimum counts were also found at every pH as follows: 33 samples at a pH of 3.4, 8 at a pH of 3.8, 2 at a pH of 4.2, and 5 at a pH of 4.6, with one sample having the same count at pH values of 3.4 and 4.6, and another at pH values of 3.4, 3.8 and 4.2.

While maximum and minimum counts were found at all pH values of the medium, the general trend of the results showed that the number of yeast colonies decreased slightly as the pH of the medium increased. This is more clearly shown in the average number of yeast colonies and the average estimated yeast counts for each pH value of the medium as summarized in table 3.

TABLE 3

The number of yeast colonies from butter samples for each pH value of the medium

SAMPLE NUMBER	DILUTION	pH 3.0	pH 3.4	pH 3.8	pH 4.2	pH 4.6
1	1:1,000	19.0	35.5	36.5	45.5	59.0
2	1:100	142.5	156.5	179.0	189.0	183.0
3	1:100	343.5	393.5	425.0	411.5	455.0
4	1:100	13.0	17.5	21.0	17.0	13.5
5	1:1,000	44.0	51.0	55.5	63.5	67.0
6	1:1,000	131.0	141.0	148.5	144.0	135.5
7	1:10	69.5	122.0	170.0	185.5	180.5
10	1:100	95.0	81.0	69.0	83.5	99.0
11	1:10	7.0	6.5	9.5	13.0	12.5
12	1:10	5.5	7.0	9.0	11.5	10.5
13	1:10	2.5	4.5	4.5	4.5	5.0
14	1:100	126.0	151.5	179.5	174.5	195.0
15	1:100	11.0	18.0	26.5	34.0	31.0
16	1:100	20.0	21.5	32.0	35.0	31.0
17	1:100	59.0	56.0	66.5	85.0	81.5
18	1:100	18.5	21.5	25.0	31.0	35.0
19	1:100	17.5	15.5	21.0	16.0	23.0
20	1:10	132.5	133.0	133.5	147.0	124.5
21	1:10	10.0	24.5	24.0	29.0	34.0
22	1:10	2.0	4.0	6.5	4.5	6.0
23	1:10	286.0	410.5	440.5	462.0	445.5
24	1:100	47.5	65.0	72.0	69.5	68.0
25	1:100	35.0	47.5	66.0	62.0	53.0
26	1:10	7.0	13.0	22.0	27.0	30.5
27	1:10	17.0	46.0	42.5	39.0	52.0
28	1:10	14.5	23.5	36.5	38.0	39.5
29	1:10	48.0	45.0	71.0	83.5	73.0
30	1:1,000	236.5	217.5	241.5	252.5	291.5
31	1:100	13.5	35.5	61.5	71.5	74.5
32	1:100	60.0	76.5	77.0	85.0	84.0
33	1:10	19.5	21.5	20.0	22.0	26.0
34	1:10	165.0	187.0	179.5	189.0	186.5
35	1:100	38.5	41.5	59.5	52.5	49.5
36	1:100	49.0	44.0	58.0	61.5	67.0
37	1:100	34.5	34.0	33.0	46.5	55.5
38	1:100	13.0	28.0	28.5	32.0	28.0
39	1:100	24.0	28.0	30.5	34.0	31.0
40	1:10	52.0	67.0	90.0	102.5	118.5
41	1:100	587.5	692.0	692.5	692.0	671.5
42	1:10	207.5	227.5	252.5	235.5	258.5
43	1:10	193.5	302.0	288.5	307.5	290.5
46	1:100	262.0	280.5	298.5	297.0	283.5
47	1:1,000	39.0	45.0	54.0	66.5	63.0

TABLE 3—*Concluded*

SAMPLE NUMBER	DILUTION	pH 3.0	pH 3.4	pH 3.8	pH 4.2	pH 4.6
48	1:1,000	9.5	16.5	17.5	15.0	20.5
49	1:100	33.0	32.0	30.5	34.0	30.0
50	1:100	96.0	90.0	113.5	117.0	108.0
52	1:100	4.0	6.0	7.0	14.0	11.5
53	1:100	112.0	132.5	121.0	130.0	124.0
54	1:100	375.5	403.5	453.0	438.5	471.0
55	1:100	31.5	41.5	40.0	45.5	53.5
Averages 50 samples....		87.6	103.2	112.8	116.9	118.8
Estimated average yeast count.....		15,153	16,480	18,003	18,838	19,930

Although these figures show lower average yeast counts at the lower pH values of the medium, the variations in the counts at pH values of 3.4 to 4.6 are not great enough to be of practical significance in routine analysis of butter. The maximum average number of colonies per plate at pH 4.6 was only 13.5 per cent greater than the minimum average at a pH of 3.4, and the variation between the maximum and minimum estimated yeast counts per cubic centimeter of butter was approximately 17 per cent.

For individual samples, the greatest percentage difference in the counts was for sample 26, the minimum count being 57.4 per cent lower than the maximum count, while the smallest variation was 3.1 per cent for sample 41. The average variation for all samples was approximately 27 per cent. In general the widest percentage variations in the counts occurred in the case of samples where there were comparatively few colonies appearing on the plates due to using too high dilutions or to samples being moderately low count butter.

In table 4 is shown the distribution of the samples of butter according to the percentage variation in the counts when the medium was acidified to pH values of 3.4 to 4.6.

These figures show that 42 samples, or 84 per cent, had a variation of less than 40 per cent between maximum and minimum counts, with only 8 samples having variations of over 40 per cent.

As previously mentioned in the outline of the methods of this

study, all plates from butter samples were examined for bacterial colonies, and a microscopic examination made of stained smears of twelve representative colonies from a selected area of the plate. This examination showed that, in the case of 40 samples of butter, bacterial colonies were easily distinguishable at pH 4.2 and 4.6. Many of the plates were crowded with bacterial colonies, while in others only a few were evident.

With 14 samples of butter some of the subsurface colonies that were counted as yeasts were found to be bacteria on a microscopic examination of the smears. In all cases, with one exception, not more than two bacterial colonies were found in the twelve that were examined. On none of the plates were bacterial colonies evident at pH 3.0, 3.4 or 3.8, nor were any bacteria found on the examination of the smears under the microscope.

TABLE 4

The distribution of butter samples according to the percentage variation in the yeast counts at different pH values

PER CENT VARIATION	NUMBER OF SAMPLES	PER CENT OF SAMPLES
Less than 10	7	14
10–20	11	22
20–30	10	20
30–40	14	28
40–50	4	8
Over 50	4	8
Total samples.....	50	100

As with the pure yeast cultures all plates were carefully examined to determine if the pH of the medium made any noticeable difference in the size of the yeast colonies. There was no apparent difference in the size of the surface colonies at any pH, and the colonies that were present on the medium of pH 3.0 and 3.4 grew and developed as well as at the higher pH values.

The number of moulds appearing on the plates were very few, many of the samples of butter showing no mould growth at any pH of the medium. For the samples that showed mould growth, there was practically no difference in the numbers developing on the plates at any pH.

DISCUSSION OF RESULTS

From the results obtained in the study of representative yeasts isolated from Canadian pasteurized butter, it was apparent that all cultures were able to grow equally well over a range of pH values from 3.4 to 4.6 which represented the range of pH of the media commonly used for routine mould and yeast analysis of butter, while the majority of samples were able to make good growth even at a pH of 3.0.

These results showed that a pH of approximately 3.5± had no detrimental effect on the development of butter yeasts, and as there was no apparent difference in size of colonies or rapidity of growth, the data indicated that a medium of pH 3.5± would give as satisfactory yeast counts from butter as a medium of pH 4.2 or 4.6 which is commonly used by some workers.

For the butter samples, while the general trend of the results was for the number of yeast colonies to decrease as the pH of the medium increased, the data reported do not show differences in the counts great enough to be considered significant in the routine analysis of butter for moulds and yeasts.

Furthermore, the decrease in the counts was not consistent for individual cultures and maximum and minimum counts were obtained at all pH values, excluding the counts at pH 3.0. The average variation in the counts of individual samples and the difference in the average counts at each pH were less than found by several workers (6, 7, 8) in making a series of plate counts of the same sample of milk and using the same medium. This is also true in regard to the results from pure yeast cultures and would indicate that the variations in the yeast counts as shown by these data were probably due more to technique and personal factors than to the difference in the pH of the medium.

The results also show that when the medium had a pH of 4.2 and 4.6, bacterial colonies were always present on the plates, while at pH 3.0, 3.4, and 3.8 no bacterial colonies were evident although Hood and White (3) in previous work found a few bacteria when media had a pH of 3.8. Even when the plates at pH values of 4.2 and 4.6 were carefully examined with a hand

lens, and in some cases with the low power lens of a microscope, some bacterial colonies were counted as yeasts and were only distinguishable by a microscopic examination of stained smears. This fact, no doubt, increased to some extent the counts obtained at pH 4.2 and 4.6.

Such a time consuming practice as the examination of colonies under the low power lens of a micrsocope and of making stained smears of doubtful colonies would be impractical in the control laboratory making a routine analysis for moulds and yeasts of a great many samples of butter per day.

When the medium is acidified to a pH of 3.5± the growth of bacteria on the plates is inhibited and the laboratory worker is reasonably sure that all colonies are yeasts. This not only makes for greater ease and quickness in counting but eliminates the error due to the inclusion of bacterial colonies, which would tend to give greater uniformity to mould and yeast counts when made by different workers. This is important in Canada where results from different laboratories are used for comparative purposes and where such counts are taken into consideration in scoring exhibition butter.

In this connection it is interesting to note that while the yeast counts for butter samples at pH 3.4 and 3.8 were slightly lower in general than at pH 4.2 and 4.6 there was not a sufficient difference to change the rating of the count according to the classification (9) as used in Canada, nor to change the numerical score given for mould and yeast counts by the modified score card (1) for butter. Thus there is no advantage in using a pH of 4.2 or 4.6 for mould and yeast work and there is the decided disadvantage of having bacterial colonies on the plates at the higher pH values which tends to introduce errors in the counts.

SUMMARY AND CONCLUSIONS

The data presented show that for pure cultures of yeasts there was no significant differences in the average counts or the counts of individual cultures at all of the pH values used. Maximum and minimum counts of individual cultures were obtained at all pH values. For butter samples, the data showed that in a general

way the yeast counts decreased slightly as the pH of the medium increased but the differences in the counts were insignificant at pH values of 3.4 to 4.6 for routine control work.

At pH values of 3.4 and 3.8 no bacterial colonies were found on the plates. For the majority of the butter samples, however, many bacterial colonies were evident at pH values of 4.2 and 4.6. Although the majority of the bacterial colonies were easily distinguishable some were included in the counts as yeasts even after a careful examination of the plates, which introduced errors in the counts.

The pH of the medium had no apparent effect on the size or rapidity of growth of the yeast cultures from the pure cultures of yeasts and from butter.

While few samples had mould counts high enough to obtain much comparative information, there was no indication that the growth of moulds was affected by the pH values of the medium used in the study.

As the data show that pH values of 3.4 to 3.8 in the medium effectively inhibits the growth of bacterial colonies, but does not affect significantly the mould and yeast counts of butter, a pH of 3.5± which allows for a margin of safety in standardizing the medium is considered to be most satisfactory for the routine analysis of butter for moulds and yeasts.

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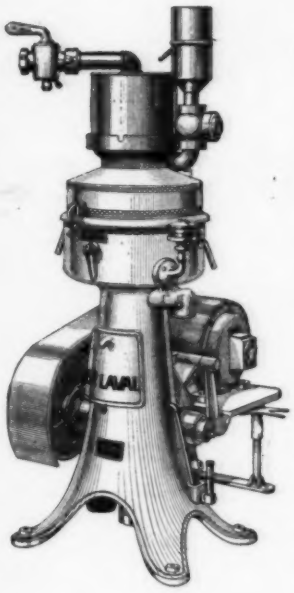

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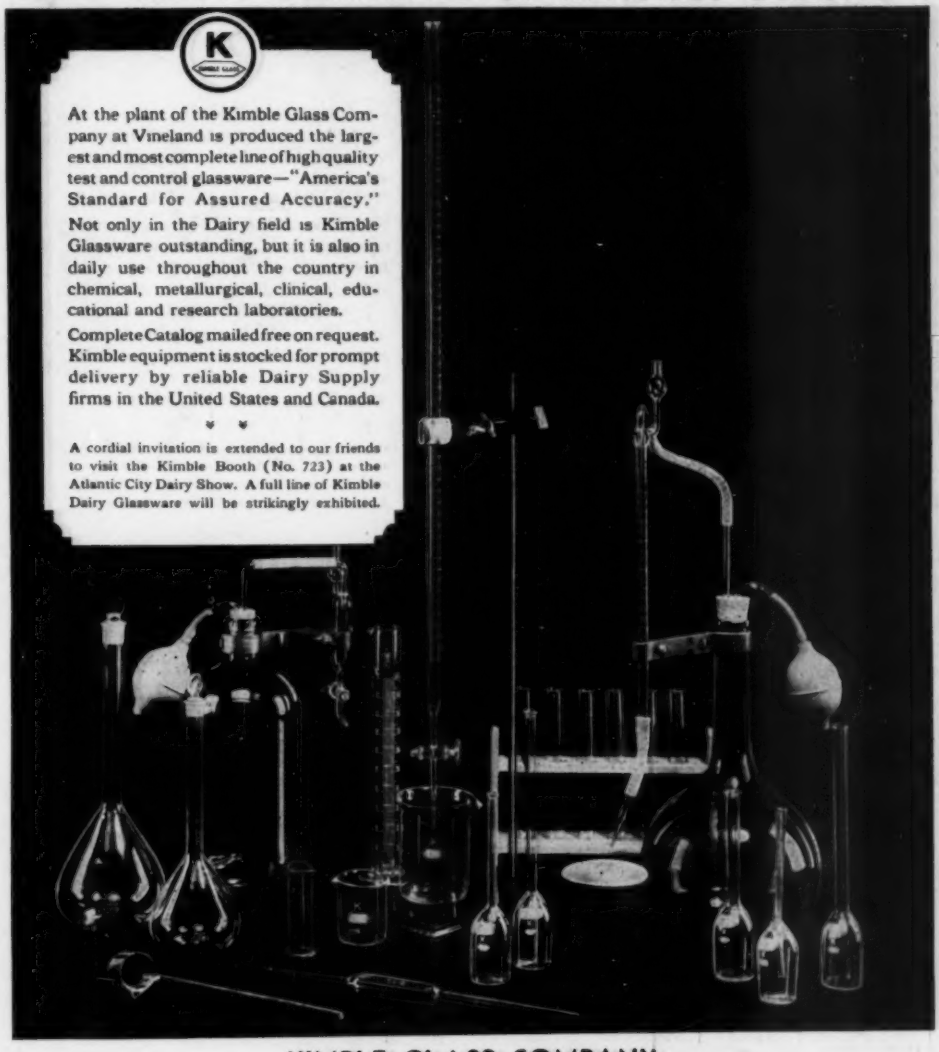

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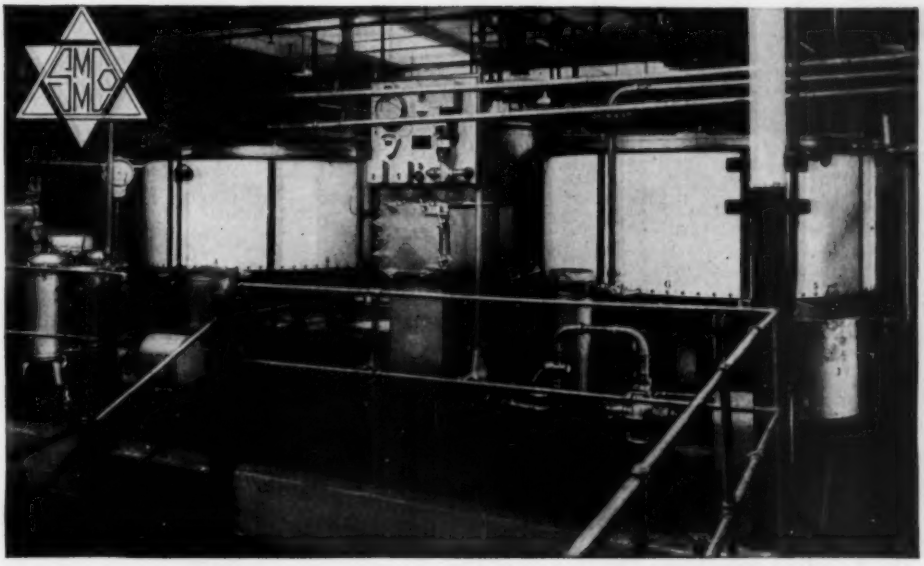

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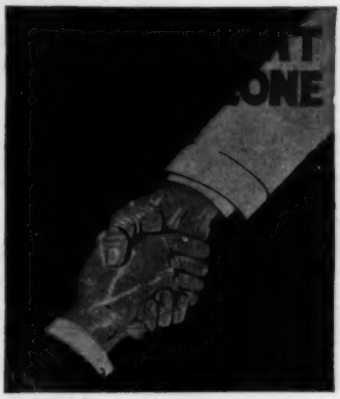

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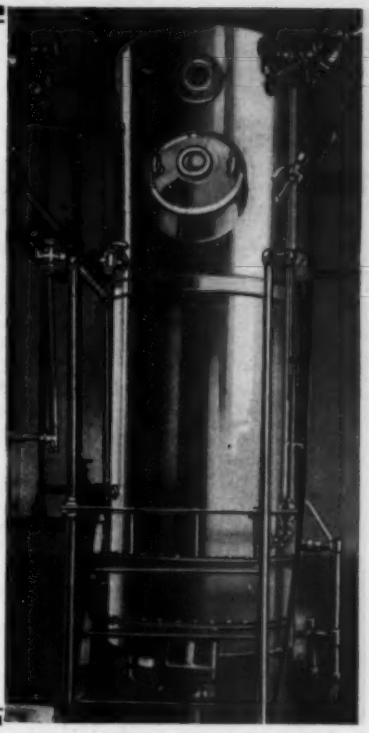

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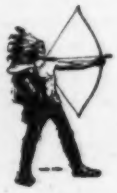

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